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(54) Title: COMPOSITIONS AND METHODS FOR INHIBITING G2 CELL CYCLE ARREST AND SENSITIZING CELLS TO DNA DAMAGING AGENTS

(57) Abstract: The invention provides compositions and methods for inhibiting Chk1 and/or Chk2 kinases. Also provided are compositions and methods for inhibiting G2 cell arrest checkpoint, particularly in mammalian, e.g., human, cells. The compositions and methods of the invention are also used to treat disorders of cell growth, such as cancer. In particular, the invention provides methods for selectively sensitizing G1 checkpoint impaired cancer cells to DNA damaging agents and treatments. Also provided are methods for screening for compounds able to interact with, e.g., inhibit, enzymes involved in the G2 cell cycle arrest checkpoint, such as Chk1 and/or Chk2/Cds1 kinase.

COMPOSITIONS AND METHODS FOR INHIBITING G2 CELL CYCLE ARREST AND SENSITIZING CELLS TO DNA DAMAGING AGENTS

TECHNICAL FIELD

This invention generally pertains to the fields of medicine and cancer therapeutics. In particular, this invention provides novel genes and polypeptides and methods for making and using them. Specifically, the compositions and methods of the invention are used to treat disorders of cell growth, such as cancer. In particular, the invention provides methods for selectively sensitizing G1 checkpoint impaired cancer cells to DNA damaging agents and treatments. Also provided are methods for screening for compounds able to interact with, e.g., inhibit, enzymes involved in the G2 cell cycle arrest checkpoint, such as Chk1 and/or Chk2/Cds1 kinase.

BACKGROUND

It is a continuing challenge to develop anti-cancer agents that are capable of inhibiting the growth of, or killing, cancer cells, without affecting normal cells. Researchers have focused on genetic mutations in cancer cells to find clues to discover such new anti-cancer drugs.

Many cancer cells have mutations in genes involved in the G1 cell cycle arrest checkpoint. Such genes include impaired tumor suppressor genes, e.g., p53, Rb, $p16^{INK4}$, and $p19^{ARF}$. Alternatively, such mutations can cause expression of oncogenes, e.g., MDM-2 and cyclin D. In addition to these, excessive growth factor signaling can be caused by the over expression of growth factors. Together with these gain-of-function mutations, growth factor receptors or downstream signal-transducing molecules can cause cell transformation by overriding the G1 checkpoint. In contrast, few cancers have disrupted G2 cell cycle arrest checkpoints. Thus, the G2 checkpoint is usually retained in cancer cells with the impaired G1 checkpoint.

If the G2 checkpoint could be selectively disrupted, cancer cells with an impaired G1 checkpoint would become more sensitive to DNA-damaging treatment, as compared to normal cells (with intact G1), since progression through G1 and G2 without repairing such damage induces apoptosis.

The mechanism that promotes the cell cycle G2 arrest after DNA damage is conserved among species from yeast to human. In the presence of damaged DNA, Cdc2/Cyclin B kinase is kept inactive because of inhibitory phosphorylation of threonine-14 and tyrosine-15 residues on Cdc2 kinase. At the onset of mitosis, the dual phosphatase Cdc25 kinase removes these inhibitory phosphates and thereby activates Cdc2/Cyclin B kinase.

In fission yeast, the protein kinase Chk1 is required for the cell cycle arrest in response to damaged DNA. Chk1 kinase acts downstream of several rad gene products and is modified by the phosphorylation upon DNA damage. The kinases Rad53 of budding yeast and Cds1 of fission yeast are known to conduct signals from unreplicated DNA. It appears that there is some redundancy between Chk1 and Cds1 because elimination of both Chk1 and Cds1 was culminated in disruption of the G2 arrest induced by damaged DNA. Interestingly, both Chk1 and Cds1 phosphorylate Cdc25 kinase and promote Rad24 binding to Cdc25, which sequesters Cdc25 to cytosol and prevents Cdc2/Cyclin B activation. Therefore Cdc25 appears to be a common target of theses kinases and presumably an indispensable factor in the G2 checkpoint.

In humans, both hChk1, a human homologue of fission yeast Chk1, and Chk2/HuCds1, a human homologue of the budding yeast Rad53 and fission yeast Cds1, phosphorylate Cdc25C at serine-216, a critical regulatory site, in response to DNA damage. This phosphorylation creates a binding site for small acidic proteins 14-3-3s, human homologues of Rad24 and Rad25 of fission yeast (Lopez-Girona (1999) Nature 397:172-175). The regulatory role of this phosphorylation was clearly indicated by the fact that substitution of serine-216 to alanine on Cdc25C disrupted cell cycle G2 arrest in human cells (Peng (1997) Science 277:1501-1505).

SUMMARY

This invention provides nucleic acids and polypeptides which can be used to treat cell proliferative disorders, such as those associated with benign and malignant tumor cells. While the invention is not limited to any particular mechanisms, the polypeptides of the invention can function by inhibiting the G2 cell cycle arrest checkpoint. Thus, the

invention also provides compositions and methods for selectively sensitizing a cell with an impaired G1 cell cycle arrest checkpoint, e.g., a cancer cell, to a DNA damaging agent

The invention provides an isolated or recombinant polypeptide comprising the amino acid sequence: $X_1 X_2 X_3 X_4 X_5 X_6 X_7 X_8 X_9 X_{10} X_{11}$, wherein X1 is L, F, W, M, R, I, V, Y, K, or absent, X2 is Y, F, A, W, S or T, X3 is any amino acid, X4 is any amino acid, X5 is any amino acid, X6 is S, A, N, H or P, X7 is any amino acid, X8 is any amino acid, X9 is any amino acid or absent, X10 is N, G, L, S, M, P, N, A or absent, and X11 is L or absent, wherein the polypeptide when administered to or expressed in a cell disrupts the G2 cell cycle arrest checkpoint.

In alternative embodiments, for the isolated or recombinant polypeptide of the invention: X_1 is L, F, W, M, R or absent or X_1 is L, F or W; X_2 is Y, F, A; X_3 is R, T, S, H, D, G, A, L, K, A, N, Q or P, or, X_3 is R, T, S, H, D, G, A or L, or, X_3 is R, T, S or H; X_4 is S, T, G, A, L, R, I, M, V, P, or, X_4 is S, T, G, A, L, R, or, X_4 is S; X_5 is P, A, G, S or T, or, X_5 is P; X_6 is S, N, H, P, A, G or T, or, X_6 is S, N or H, or, X_6 is S; X_7 is M, F, Y, D, E, N, Q, H, G, I, L, V, A, P, N or W, or, X_7 is M, F, Y, D, E, N, Q or H, or, X_7 is M, F, Y, Q or H; X_8 is P, F, Y, W, L, G, M, D, E, N, Q, H, I, V, A or P, or, X_8 is P, F, Y or W, or, X_8 is Y; X_9 is E, G, L, S, M, P, N, D, A, T, P or absent; X_{10} is absent; X_{11} is absent.

In one embodiment, the invention provides a polypeptide wherein X_2 is Y, X_5 is P, and X_{10} is N. In one embodiment, the invention provides a polypeptide wherein X_3 is R, X_8 is P, and X_{11} is L. In one embodiment, the invention provides a polypeptide wherein X_4 is S, X_5 is P, X_6 is S, X_9 is E, X_{10} is N and X_{11} is L.

In alternative embodiments, the invention provides an isolated or recombinant polypeptide wherein the amino acid sequence comprises Y G G P G G G G N; R Y S L P P E L S N M; L A R S A S M P E A L; L Y R S P S M P E N L; L Y R S P A M P E N L; W Y R S P S F Y E N L; W Y R S P S Y Y E N L; or, W Y R S P S Y Y.

In alternative embodiments, the invention provides an isolated or recombinant polypeptide wherein the amino acid sequence comprises L Y R S P S Y P E N L, L Y R S P S Y F E N L, L Y R S P S Y Y E N L, or L Y R S P S Y W E N L.

In alternative embodiments, the invention provides an isolated or recombinant polypeptide wherein the amino acid sequence comprises L Y R S P S N P E N L, L Y R S P S N F E N L, L Y R S P S N Y E N L, or L Y R S P S N W E N L.

In alternative embodiments, the invention provides an isolated or recombinant polypeptide wherein the amino acid sequence comprises L Y R S P S H P E N L, L Y R S P S H F E N L, L Y R S P S H Y E N L, L Y R S P S H W E N L, L Y S S P S M P E N L, L Y S S P S M F E N L, L Y S S P S M Y E N L, L Y S S P S M W E N L, L Y S S P S F P E N L, L Y S S P S F P E N L, L Y S S P S F F E N L, L Y S S P S F Y E N L, L Y S S P S F W E N L, L Y S S P S Y P E N L, L Y S S P S Y F E N L, L Y S S P S Y Y E N L, or L Y S S P S Y W E N L.

In alternative embodiments, the invention provides an isolated or recombinant polypeptide wherein the amino acid sequence comprises L Y S S P S Q P E N L, L Y S S P S Q W E N L, L Y S S P S H P E N L, L Y S S P S H F E N L, L Y S S P S H Y E N L, L Y S S P S H W E N L, L Y T S P S M P E N L, L Y T S P S M F E N L, L Y T S P S M Y E N L, L Y T S P S M W E N L, L Y T S P S F P E N L, L Y T S P S F F E N L, L Y T S P S F Y E N L, L Y T S P S F W E N L, L Y T S P S Y P E N L, L Y T S P S Y F E N L, L Y T S P S Y Y E N L, or L Y T S P S Y W E N L.

In alternative embodiments, the invention provides an isolated or recombinant polypeptide wherein the amino acid sequence comprises L Y T S P S N P E N L, L Y T S P S N F E N L, L Y T S P S N Y E N L or L Y T S P S N W E N L.

In alternative embodiments, the invention provides an isolated or recombinant polypeptide wherein the amino acid sequence comprises L Y T S P S H P E N L, L Y T S P S H F E N L, L Y T S P S H Y E N L or L Y T S P S H W E N L.

In alternative embodiments, the invention provides an isolated or recombinant polypeptide wherein the amino acid sequence comprises L Y H S P S Y P E N L, L Y H S P S Y F E N L, L Y H S P S Y Y E N L or L Y H S P S Y W E N L.

In alternative embodiments, the invention provides an isolated or recombinant polypeptide wherein the amino acid sequence comprises L F T S P S Y P E N L, L F T S P S Y F E N L, L F T S P S Y Y E N L or L F T S P S Y W E N L.

In alternative embodiments, the invention provides an isolated or recombinant polypeptide wherein the amino acid sequence comprises F Y S S P S H P E N L, F Y S S P S H F E N L, F Y S S P S H Y E N L, F Y S S P S H W E N L, F Y T S P S M P E N L, F Y T S P S M F E N L, F Y T S P S M Y E N L, F Y T S P S M W E N L, F Y T S P S F P

E N L, F Y T S P S F F E N L, F Y T S P S F Y E N L, F Y T S P S F W E N L, F Y T S P S Y P E N L, F Y T S P S Y F E N L, F Y T S P S Y Y E N L or F Y T S P S Y W E N L.

In alternative embodiments, the invention provides an isolated or recombinant polypeptide wherein the amino acid sequence comprises W Y R S P S M P E N L, W Y R S P S M F E N L, W Y R S P S M Y E N L, W Y R S P S M W E N L, W Y R S P S F P E N L, W Y R S P S F F E N L, W Y R S P S F Y E N L, W Y R S P S F W E N L, W Y R S P S Y P E N L, W Y R S P S Y F E N L, W Y R S P S Y Y E N L or W Y R S P S Y W E N L.

In alternative embodiments, the invention provides an isolated or recombinant polypeptide wherein the amino acid sequence comprises W Y T S P S M P E N L, W Y T S P S M F E N L, W Y T S P S M Y E N L, W Y T S P S M W E N L, W Y T S P S F P E N L, W Y T S P S F F E N L, W Y T S P S F Y E N L, W Y T S P S F W E N L, W Y T S P S Y P E N L, W Y T S P S Y F E N L, W Y T S P S Y Y E N L or W Y T S P S Y W E N L.

In alternative embodiments, the invention provides an isolated or recombinant polypeptide wherein the amino acid sequence comprises W Y T S P S H P E N L, W Y T S P S H F E N L, W Y T S P S H Y E N L or W Y T S P S H W E N L.

In alternative embodiments, the invention provides an isolated or recombinant polypeptide wherein the amino acid sequence comprises L K R S P S M P E N L, L Y I S P S M P E N L or L Y R S P S M V E N L.

In one embodiment, the invention provides an isolated or recombinant polypeptide wherein the polypeptide when administered to or expressed in a cell disrupts the G2 cell cycle arrest checkpoint, wherein the cell is a mammalian cell. The cell can be a human cell, a yeast cell, an insect cell, a bacterial cell, a plant cell, and the like.

In one embodiment, the invention provides an isolated or recombinant polypeptide further comprising a cell membrane permeant. The cell membrane permeant can comprise a polypeptide, such as a TAT protein transduction domain, e.g., comprising a sequence Y G R K K R R Q R R R. Alternatively, the cell membrane permeant can comprise a lipid, such as a liposome.

The invention provides a chimeric polypeptide comprising a first domain comprising a polypeptide of the invention and a second domain comprising a cell membrane

permeant, wherein the polypeptide when administered to or expressed in a cell disrupts the G2 cell cycle arrest checkpoint. The chimeric polypeptide can be a recombinant fusion protein.

The invention provides an isolated or recombinant nucleic acid encoding a polypeptide or a chimeric polypeptide of the invention, wherein the polypeptide, when administered to or expressed in a cell, disrupts the G2 cell cycle arrest checkpoint.

The invention provides an expression vector comprising a nucleic acid encoding a polypeptide or a chimeric polypeptide of the invention, wherein the polypeptide, when administered to or expressed in a cell, disrupts the G2 cell cycle arrest checkpoint.

The invention provides a cell comprising a nucleic acid or an expression vector of the invention. The cell can be a bacterial, a yeast, an insect, a plant, or a mammalian cell.

The invention provides a pharmaceutical composition comprising a polypeptide of the invention, a nucleic acid of the invention, an expression vector of the invention, or a cell of the invention; and, a pharmaceutically acceptable excipient. In one embodiment, the pharmaceutical composition can comprise a liposome.

The invention provides a method for inhibiting a the activity of a Chk1 kinase or a Chk2 kinase comprising contacting the kinase with a polypeptide of the invention or a pharmaceutical composition of the invention, in an amount sufficient to inhibit the activity of the Chk1 or Chk2 kinase.

The invention provides a method for disrupting a cell G2 cell cycle arrest checkpoint comprising contacting the cell with a polypeptide of the invention or a pharmaceutical composition of the invention in an amount sufficient to disrupt the G2 cell cycle arrest checkpoint. In alternative embodiments the cell is a mammalian cell, a human cell or a cancer cell.

The invention provides a method for sensitizing a cell to a DNA damaging agent comprising contacting the cell with a polypeptide of the invention or a pharmaceutical composition of the invention in an amount sufficient to disrupt the G2 cell cycle arrest checkpoint, thereby sensitizing the cell to the DNA damaging agent. In alternative embodiments the cell is a mammalian cell, a human cell or a cancer cell. The cancer cell can have an impaired G1 cell cycle arrest checkpoint.

The invention provides a method for selectively sensitizing a cell with an impaired G1 cell cycle arrest checkpoint to a DNA damaging agent comprising contacting the cell with a polypeptide of the invention or a pharmaceutical composition of the invention, in an amount sufficient to disrupt the G2 cell cycle arrest checkpoint, thereby sensitizing the cell to the DNA damaging agent. In alternative embodiments the cell is a mammalian cell, a human cell or a cancer cell.

The invention provides a method for inducing apoptosis in a cell in an individual comprising a administering a polypeptide of the invention or a pharmaceutical composition of the invention, in an amount sufficient to disrupt the G2 cell cycle arrest checkpoint in the cancer cell, thereby sensitizing the cancer cell to a DNA damaging agent, and administering a DNA damaging agent. In alternative embodiments the cell is a mammalian cell, a human cell or a cancer cell. The cancer cell can have an impaired G1 cell cycle arrest checkpoint. The DNA damaging agent can be 5-fluorouracil (5-FU), rebeccamycin, adriamycin, bleomycin, cisplatin, hyperthermia, UV irradiation or gamma-irradiation.

The invention provides a method for screening for compounds capable of modulating the activity of a Chk1 kinase or a Chk2 kinase comprising the following steps: (a) providing a test compound; (b) providing a Chk1 kinase or a Chk2 kinase; (c) providing a polypeptide of the invention, wherein the polypeptide binds to the Chk1 kinase or the Chk2 kinase; and, (d) contacting the test compound with the kinase and the polypeptide and measuring the ability of the test compound to prevent binding of the polypeptide to the kinase.

The invention provides a method for screening for compounds capable of modulating the activity of a Chk1 kinase or a Chk2 kinase comprising the following steps: (a) providing a test compound; (b) providing a Chk1 kinase or a Chk2 kinase; (c) providing a polypeptide of the invention, wherein the polypeptide is phosphorylated by the Chk1 kinase or the Chk2 kinase; and, (d) contacting the test compound with the kinase and the polypeptide and measuring the ability of the test compound to inhibit or abrogate phosphorylation of the polypeptide by the kinase. The method can further comprising providing a full length human Cdc25C. In one embodiment of the method, the polypeptide of step (c) comprises amino acid residue serine 216 of human Cdc25C, such as comprising

from about amino acid residue 200 to about amino acid residue 250 of human Cdc25C. In one embodiment of the method, the polypeptide of step (c) further comprises glutathione-S-transferase.

In one embodiment of the methods of the invention, including the screening methods, the polypeptide of the invention is immobilized.

The invention provides a method for screening for compounds capable of specifically inhibiting the G2 cell cycle checkpoint comprising the following steps: (a) providing a test compound and a polypeptide of the invention; (b) providing a G1 checkpoint impaired cell; (c) contacting the cell of step (b) with the test compound or the polypeptide of step (a) plus a DNA damaging treatment, such as 5-fluorouracil (5-FU), rebeccamycin, adriamycin, bleomycin, cisplatin, hyperthermia, UV irradiation or gamma-irradiation, or, or an M phase checkpoint activator; and, (d) measuring the amount of DNA in the cells after the contacting of step (c) to determine if the test compound has inhibited the G2 cell cycle checkpoint, wherein the polypeptide of step (a) acts as a G2-checkpoint-inhibiting positive control. In alternative embodiments the cell is a mammalian cell, a human cell or a cancer cell. In one embodiment, the amount of DNA is measured using propidium iodide by, e.g., a FACS analysis, or equivalent. In one embodiment, the amount of DNA is measured after about 10 to about 72 hours after the contacting of step (c).

In one embodiment, the method comprises contacting the cell of step (b) with an M phase checkpoint activator alone (as a substitute for a DNA damaging agent) and the test compound or the polypeptide of step (a), wherein a test compound that has not inhibited or abrogated the arrest at the M phase checkpoint of the cell cycle after contacting the cell with an M phase activator is a specific inhibitor of the G2 cell cycle checkpoint (because it did not affect M phase checkpoint or it was not a non-specific phenomenon). In one embodiment, the M phase checkpoint activator is colchicine or nocodazole.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

All publications, patents, patent applications, GenBank sequences and ATCC deposits, cited herein are hereby expressly incorporated by reference for all purposes.

DESCRIPTION OF DRAWINGS

Figure 1 shows chimeric peptides used in and results of experiments demonstrating that TAT-S216A and TAT-S216 peptides inhibit hChk1 and Chk2/HuCds1 kinase activity *in vitro*, as described in Example 1, below. Figure 1A shows a schematic diagram of the fusion/chimeric peptides TAT-control, TAT-S216A and TAT-S216. Figure 1B shows SDS-PAGE autoradiograms demonstrating the results of *in vitro* Cdc25C phosphorylation assays using TAT-S216A and TAT-S216 peptides to inhibit purified hChk1 activity; amino acid residues 200 to 256 of Cdc25C (SEQ ID NO:1) were used as a substrate at a concentration of 1 μM. Figure 1C shows SDS-PAGE autoradiograms demonstrating the results of *in vitro* Cdc25C phosphorylation assays using TAT-S216A peptide to inhibit purified hChk1 and Chk2/HuCds1 activity; amino acid residues 211 to 220 of Cdc25C (SEQ ID NO:1) were used as a substrate at a concentration of 10 μM.

Figure 2 the results of experiments demonstrating that TAT-S216A and TAT-S216 peptides can abrogate DNA damage-induced G2 arrest in Jurkat cells. Figure 2A shows the results of a FACS analysis of Jurkat cells treated with bleomycin (10 μg/ml) and TAT-S216A and TAT-S216 peptides (10 μM each). Figure 2B shows the results of an SDS-PAGE of cell lysates from a histone H1 kinase analysis; lysates were prepared from cells treated with the indicated reagent for six hours. Figure 2C shows the results a FACS analysis of colchicines- (5 μg/ml) and peptide- (10 μM each) treated cells; Jurkat cells were treated for 20 hours.

Figure 3 shows the results of experiments demonstrating that TAT-S216A and TAT-S216 peptides can specifically sensitize cancer cells to bleomycin, but not colchicine. Figure 3A shows the results of trypan blue dye exclusion analysis of Jurkat cells treated with bleomycin with or without the TAT-S216A and TAT-S216 peptides. Figure 3B shows the results of trypan blue dye exclusion (survival) analysis of Jurkat cells treated with colchicine with or without the TAT-S216A and TAT-S216 peptides. Figure 3C shows the results of trypan blue dye exclusion (survival) analysis of PHA blasts treated with bleomycin with or without the TAT-S216A and TAT-S216 peptides. Figure 3D shows the results of FACS analysis PHA blasts treated with bleomycin with or without the TAT-S216A and TAT-S216 peptides (vertical axis is DNA content indicated by propidium iodide staining).

Figure 4 shows the results of experiments demonstrating that TAT-S216A and TAT-S216 peptides can sensitize cancer cells to bleomycin. Figure 4A shows the results of X-TT analysis of PANC1 cells treated with bleomycin with or without the TAT-S216A and TAT-S216 peptides. Figure 4B shows the results of X-TT analysis of MIA PaCa2 cells treated with bleomycin with or without the TAT-S216A and TAT-S216 peptides.

Figure 5 shows a schematic 3-dimensional structure of human Chk2 interacting with exemplary G2-abrogating peptides of the invention, as described in Example 2, below.

Figure 6 shows the results of FACS analysis of the amount of DNA in cells to determine the number of cells in one of the four cell cycle phases after incubating these cells with bleomycin and exemplary peptides of the invention, as described in Example 3, below.

Figure 7 shows the results of FACS analysis of the amount of DNA in cells to determine the number of cells in one of the four cell cycle phases after incubating these cells with colchicine and exemplary peptides of the invention, as described in Example 3, below.

Figure 8 shows the sequences of peptides used in experiments described in Example 4, below.

Figure 9 shows a summary of results of experiments as described in Example 4, below.

Figure 10 shows the results of experiments demonstrating that a peptide of the invention (as a S216-containing fusion protein) administered to an animal *in vivo* effectively sensitized cancer cells to a DNA damaging agent.

Figure 11 shows the results of experiments demonstrating that a peptide of the invention (as a R-II-containing fusion protein) administered to an animal *in vivo* effectively sensitized cancer cells to a DNA damaging agent.

Like reference symbols in the various drawings indicate like elements.

DETAILED DESCRIPTION

The genes and polypeptides of the invention provide a novel means to treat cell proliferative disorders, including, e.g., to stop the growth of, or kill, cancer cells. While the invention is not limited by any particular mechanism of action, administration of the polypeptides of the invention will delay or abrogate G2 cell cycle arrest checkpoint in cells.

The genes and polypeptides of the invention can also be used to inhibit Chk1 and/or Chk2/Cds1 kinase activity. Inhibition of Chk1 and/or Chk2/Cds1 kinase may be the mechanism by which the G2 checkpoint is inhibited. The invention also provides methods for selectively sensitizing G1 checkpoint impaired cancer cells to DNA damaging agents and treatments. Also provided are methods for screening for compounds able to interact with, e.g., inhibit, enzymes involved in the G2 cell cycle arrest checkpoint, such as Chk1 and/or Chk2/Cds1 kinases. Thus, the invention provides methods to screen for compounds that inhibit or abrogate cell cycle G2 checkpoint.

The invention for the first time describes amino acid peptide motifs in the human Cdc25C (hCdc25C) polypeptide (SEQ ID NO:1) that are the substrate motifs for human Chk1 (hChk1) (SEQ ID NO:3) and human Chk2/ human Cds1 (Chk2/HuCds1) (SEQ ID NO:4) kinase activity. The kinase-inhibitory polypeptides and nucleic acids of the invention are modeled on these hCdc25C peptide motifs. Wild-type hCdc25C is phosphorylated by hChk1 (SEQ ID NO:3) and Chk2/HuCds1 (SEQ ID NO:4).

Phosphorylation of Cdc25C is necessary for the cell's arrest at G2 checkpoint. Thus, the polypeptides and peptides of the invention, by inhibiting the phosphorylation of Cdc25C (by enzymes which probably include Chk1 and Chk2/HuCds1), can inhibit or abrogate the cell's G2 checkpoint capability. The lack of an effective G2 checkpoint after DNA damage becomes fatal to the cell (see, e.g., Maity (1994) Radiother. Oncol. 31:1-13). If a cell progresses through G2 without sufficient repair of DNA damage it becomes apoptotic. Thus, the compositions of the invention can be used to sensitize cells, such as tumor cells, to DNA damaging agents. In fact, as discussed below, the compositions of the invention can sensitize cancer cells to the apoptotic effects of DNA-damaging agents with little or no cytotoxic effect on normal cells.

Example 1, below, describes the synthesis and use of two exemplary polypeptides of the invention. Two peptides corresponding to amino acids 211 to 221 of human Cdc25C (SEQ ID NO:1) fused with a part of HIV-1-TAT (SEQ ID NO:5). These peptides were demonstrated to inhibit hChk1 kinase (SEQ ID NO:3) and Chk2/HuCds1 kinase (SEQ ID NO:4) activity *in vitro* and to specifically abrogate the G2 checkpoint *in vivo*. These peptides sensitized p53-defective cancer cell lines to the apoptotic effects of DNA-damaging agents without obvious cytotoxic effect on normal cells. These results

clearly demonstrate that the polypeptides comprising the motifs of the invention can be used to specifically inhibit or abrogate the cell cycle G2 checkpoint. These results demonstrate that the compositions of the invention can be used to screen for compositions that inhibit Chk1 or Chk2 kinase activity. These results also demonstrate that the compositions of the invention can be used for cancer therapy. While the invention is not limited by any particular mechanism of action, the polypeptides and peptides of the invention can be used to target and inhibit hChk1 (SEQ ID NO:3) and Chk2/HuCds1 (SEQ ID NO:4) kinases.

DEFINITIONS

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

The term "cell membrane permeant" as used herein means any composition which, when associated with a peptide or polypeptide of the invention, or a nucleic acid of the invention, causes, or assists in, the internalization of the composition into a cell. The association can be covalent (e.g., a linking reagent, or, as a fusion protein) or non-covalent (e.g., as with liposomes). For example, in one embodiment, a cell membrane permeant domain is linked to a peptide or polypeptide of the invention as a fusion protein domain, e.g., a TAT protein transduction domain (see, e.g., Vives (1997) J. Biol. Chem. 272:16010-16017). Other cell membrane permeant domains include, e.g., the PreS2- and S-domain of the hepatitis-B virus surface antigens, see, e.g., Oess (2000) Gene Ther. 7:750-758.

The term "human Cdc25C" or "hCdc25C" as used herein means, depending on the context, the human Cdc25C polypeptide (SEQ ID NO:1) or the human Cdc25C polypeptide (SEQ ID NO:1) message (cDNA) (SEQ ID NO:2) or gene (see, e.g., Peng (1997) Science 277:1501-1505). The term also includes all functional variations of hCdc25C, including, e.g., allelic variations, functional mutations, variations with additions, deletions, substitutions that retain functional activity. A Cdc25C polypeptide that has functional activity has the same activity as wild type Cdc25C, i.e., when appropriately phosphorylated, it can act in concert with other cell cycle control polypeptides to arrest cell growth at G2 under the proper conditions, e.g., under conditions in which sufficient DNA damage has incurred to induce apoptosis if the cell passes through the G2 checkpoint.

The terms "DNA damaging treatment" or "DNA damaging agent" include any treatments or agents that will cause DNA damage to a cell, including a drug, a radiation, an environmental shock, and the like, including, e.g., hyperthermia, UV radiation or gamma-radiation, in addition to the known DNA damaging drugs, e.g., 5-fluorouracil (5-FU), rebeccamycin, adriamycin, bleomycin, cisplatin and the like.

The term "disrupt the cell cycle G2 checkpoint" or "inhibit the cell cycle G2 checkpoint" means the ability of a peptide or polypeptide of the invention to inhibit (including abrogate) a Chk1 kinase and/or Chk2 kinase activity, e.g., a mammalian kinase, such as a human Chk1 (hChk1) kinase (SEQ ID NO:3) (see, e.g., Yin (2000) Mol. Pharmacol. 57:453-459) or a human Chk2/ human Cds1 kinase (Chk2/HuCds1) (SEQ ID NO:4) (see, e.g., Hirao (2000) Science 287:1824-1827), or, to disrupt (including abrogate) the ability of a cell to arrest growth at the G2 checkpoint under appropriate conditions, e.g., where conditions in the cell otherwise would cause G2 cell cycle arrest, such as the accumulation of DNA damage by, e.g., some anti-tumor agents.

The ability of a peptide or polypeptide of the invention to modulate or inhibit a Chk1 kinase and/or a Chk2 kinase activity can be easily tested *in vitro* or *in vivo* as, for example, in the assays, or variations thereof, described in Example 1, below. A peptide or polypeptide is considered an effective inhibitor if, e.g., it binds the kinase to inhibit or abrogate kinase activity. Alternatively, a peptide or polypeptide is also considered an effective inhibitor of kinase activity if it acts as a phosphorylation substrate and prevents phosphorylation of natural substrate, e.g., wild type Cdc25C, thereby disrupt the ability of a cell to arrest growth at the G2 checkpoint under appropriate conditions.

The ability of exemplary peptides or polypeptides of the invention to disrupt the ability of a cell to arrest growth at the G2 checkpoint, i.e., to act in concert with other cell cycle control polypeptides to arrest cell growth at G2 under the proper conditions, e.g., under conditions in which sufficient DNA damage has incurred to induce apoptosis if the cell passes through the G2 checkpoint can be easily tested *in vivo*, e.g., cell culture, is demonstrated in Example 1, below

The term "expression cassette" as used herein refers to a nucleotide sequence which is capable of affecting expression of a structural gene (i.e., a protein coding sequence) in a host compatible with such sequences. Expression cassettes include at least a promoter

operably linked with the polypeptide coding sequence; and, optionally, with other sequences. e.g., transcription termination signals. Additional factors necessary or helpful in effecting expression may also be used, e.g., enhancers. "Operably linked" as used herein refers to linkage of a promoter upstream from a DNA sequence such that the promoter mediates transcription of the DNA sequence. Thus, expression cassettes also include plasmids, expression vectors, recombinant viruses, any form of recombinant "naked DNA" vector, and the like. A "vector" comprises a nucleic acid which can infect, transfect, transiently or permanently transduce a cell. It will be recognized that a vector can be a naked nucleic acid, or a nucleic acid complexed with protein or lipid. The vector optionally comprises viral or bacterial nucleic acids and/or proteins, and/or membranes (e.g., a cell membrane, a viral lipid envelope, etc.). Vectors include, but are not limited to replicons (e.g., RNA replicons, bacteriophages) to which fragments of DNA may be attached and become replicated. Vectors thus include, but are not limited to RNA, autonomous self-replicating circular or linear DNA or RNA (e.g., plasmids, viruses, and the like, see, e.g., U.S. Patent No. 5,217,879), and includes both the expression and nonexpression plasmids. Where a recombinant microorganism or cell culture is described as hosting an "expression vector" this includes both extrachromosomal circular and linear DNA and DNA that has been incorporated into the host chromosome(s). Where a vector is being maintained by a host cell, the vector may either be stably replicated by the cells during mitosis as an autonomous structure, or is incorporated within the host's genome.

The term "chemically linked" refers to any chemical bonding of two moieties, e.g., as in one embodiment of the invention, a polypeptide comprising at least two peptide motifs of the invention. Such chemical linking includes the peptide bonding of a recombinantly or *in vivo* generated fusion protein.

The term "chimeric protein" or "fusion protein" refers to a composition comprising at least one polypeptide or peptide domain or motif which is associated with a second polypeptide or peptide domain or motif. For example, in one embodiment, the invention provides an isolated or recombinant nucleic acid molecule encoding a fusion protein comprising at least two domains, wherein the first domain comprises one kinase-inhibiting or G2-checkpoint inhibiting motif and the second domain comprising a second motif with the same or similar activity (for example, on motif may have a high binding

affinity for the kinase, whilst the second motif has high kinase inhibitory activity). Additional domains can comprise a polypeptide, peptide, polysaccharide, or the like. The "fusion" can be an association generated by a peptide bond, a chemical linking, a charge interaction (*e.g.*, electrostatic attractions, such as salt bridges, H-bonding, etc.) or the like. If the polypeptides are recombinant, the "fusion protein" can be translated from a common message. Alternatively, the compositions of the domains can be linked by any chemical or electrostatic means. The chimeric molecules of the invention can also include additional sequences, *e.g.*, linkers, epitope tags, enzyme cleavage recognition sequences, signal sequences, secretion signals, and the like. Alternatively, a peptide can be linked to a carrier simply to facilitate manipulation or identification/ location of the peptide.

The term "G2 checkpoint inhibitory activity" as used herein means any amount of inhibition of the G2 checkpoint.

The term "isolated" as used herein, when referring to a molecule or composition, such as, *e.g.*, a nucleic acid or polypeptide of the invention, means that the molecule or composition is separated from at least one other compound, such as a protein, other nucleic acids (*e.g.*, RNAs), or other contaminants with which it is associated *in vivo* or in its naturally occurring state. Thus, a nucleic acid or polypeptide is considered isolated when it has been isolated from any other component with which it is naturally associated, *e.g.*, cell membrane, as in a cell extract. An isolated composition can, however, also be substantially pure. An isolated composition can be in a homogeneous state and can be in a dry or an aqueous solution. Purity and homogeneity can be determined, for example, using analytical chemistry techniques such as polyacrylamide gel electrophoresis (SDS-PAGE) or high performance liquid chromatography (HPLC). Thus, the isolated compositions of this invention do not contain materials normally associated with their *in situ* environment. Even where a protein has been isolated to a homogenous or dominant band, there can be trace contaminants which co-purify with the desired protein.

The terms "polypeptide," "protein," and "peptide" include compositions of the invention that also include "analogs," or "conservative variants" and "mimetics" or "peptidomimetics" with structures and activity that substantially correspond to the polypeptide from which the variant was derived, including, e.g., variations of the peptides

and polypeptides of the invention which can either inhibit a mammalian Chk1 and/or Chk2 kinase, or, inhibit a mammalian G2 checkpoint.

The term "pharmaceutical composition" refers to a composition suitable for pharmaceutical use, e.g., as an anti-cancer agent , in a subject. The pharmaceutical compositions of this invention are formulations that comprise a pharmacologically effective amount of a composition comprising, e.g., a peptide, polypeptide, nucleic acid, vector, or cell of the invention, and a pharmaceutically acceptable carrier.

The term "promoter" is an array of nucleic acid control sequences which direct transcription of a nucleic acid. As used herein, a promoter includes necessary nucleic acid sequences near the start site of transcription, such as, in the case of a polymerase II type promoter, a TATA element. A promoter also optionally includes distal enhancer or repressor elements which can be located as much as several thousand base pairs from the start site of transcription. A "constitutive" promoter is a promoter which is active under most environmental and developmental conditions. An "inducible" promoter is a promoter which is under environmental or developmental regulation. A "tissue specific" promoter is active in certain tissue types of an organism, but not in other tissue types from the same organism. The term "operably linked" refers to a functional linkage between a nucleic acid expression control sequence (such as a promoter, or array of transcription factor binding sites) and a second nucleic acid sequence, wherein the expression control sequence directs transcription of the nucleic acid corresponding to the second sequence.

The term "recombinant" refers to a polynucleotide synthesized or otherwise manipulated *in vitro* (*e.g.*, "recombinant polynucleotide"), to methods of using recombinant polynucleotides to produce gene products in cells or other biological systems, or to a polypeptide ("recombinant protein") encoded by a recombinant polynucleotide. For example, recombinant peptides or polypeptides or nucleic acids can be used to practice the methods of the invention. "Recombinant means" also encompass the ligation of nucleic acids having various coding regions or domains or promoter sequences from different sources into an expression cassette or vector for expression of, *e.g.*, inducible or constitutive expression of polypeptide coding sequences in the vectors used to practice this invention.

Nucleic Acids and Expression Vectors

This invention provides novel nucleic acids, including expression vectors, for use in the treatment of uncontrolled cell growth, such as cancer, and means to make and express those nucleic acids. As the genes and vectors of the invention can be made and expressed *in vitro* or *in vivo*, the invention provides for a variety of means of making and expressing these genes and vectors. One of skill will recognize that desired levels of expression of the polypeptides of the invention can be obtained by modulating the expression or activity of the genes and nucleic acids (e.g., promoters) within the vectors of the invention. Any of the known methods described for increasing or decreasing expression or activity, including tissue-specific expression, can be used for this invention. The invention can be practiced in conjunction with any method or protocol known in the art, which are well described in the scientific and patent literature.

General Techniques

The nucleic acid sequences of the invention and other nucleic acids used to practice this invention, whether RNA, cDNA, genomic DNA, vectors, viruses or hybrids thereof, may be isolated from a variety of sources, genetically engineered, amplified, and/or expressed recombinantly. Any recombinant expression system can be used, including, in addition to bacterial cells, e.g., mammalian, yeast, insect or plant cell expression systems.

Alternatively, these nucleic acids can be synthesized *in vitro* by well-known chemical synthesis techniques, as described in, e.g., Carruthers (1982) Cold Spring Harbor Symp. Quant. Biol. 47:411-418; Adams (1983) J. Am. Chem. Soc. 105:661; Belousov (1997) Nucleic Acids Res. 25:3440-3444; Frenkel (1995) Free Radic. Biol. Med. 19:373-380; Blommers (1994) Biochemistry 33:7886-7896; Narang (1979) Meth. Enzymol. 68:90; Brown (1979) Meth. Enzymol. 68:109; Beaucage (1981) Tetra. Lett. 22:1859; U.S. Patent No. 4,458,066. Double stranded DNA fragments may then be obtained either by synthesizing the complementary strand and annealing the strands together under appropriate conditions, or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

Techniques for the manipulation of nucleic acids, such as, e.g., generating mutations in sequences, subcloning, labeling probes, sequencing, hybridization and the like are well described in the scientific and patent literature, see, e.g., Sambrook, ed.,

MOLECULAR CLONING: A LABORATORY MANUAL (2ND ED.), Vols. 1-3, Cold Spring Harbor Laboratory, (1989); CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Ausubel, ed. John Wiley & Sons, Inc., New York (1997); LABORATORY TECHNIQUES IN BIOCHEMISTRY AND MOLECULAR BIOLOGY: HYBRIDIZATION WITH NUCLEIC ACID PROBES, Part I. Theory and Nucleic Acid Preparation, Tijssen, ed. Elsevier, N.Y. (1993).

Nucleic acids, vectors, capsids, polypeptides, and the like can be analyzed and quantified by any of a number of general means well known to those of skill in the art. These include, e.g., analytical biochemical methods such as NMR, spectrophotometry, radiography, electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), and hyperdiffusion chromatography, various immunological methods, e.g. fluid or gel precipitin reactions, immunodiffusion, immuno-electrophoresis, radioimmunoassays (RIAs), enzyme-linked immunosorbent assays (ELISAs), immuno-fluorescent assays, Southern analysis, Northern analysis, dot-blot analysis, gel electrophoresis (e.g., SDS-PAGE), nucleic acid or target or signal amplification methods, radiolabeling, scintillation counting, and affinity chromatography. Amplification methods include, *e.g.*, polymerase chain reaction, PCR (PCR PROTOCOLS, A GUIDE TO METHODS AND APPLICATIONS, ed. Innis, Academic Press, N.Y. (1990) and PCR STRATEGIES (1995), ed. Innis, Academic Press, Inc., N.Y., ligase chain reaction (LCR) (see, e.g., Wu (1989) Genomics 4:560; Landegren (1988) Science 241:1077; Barringer (1990) Gene 89:117); transcription amplification (see, e.g., Kwoh (1989) Proc. Natl. Acad. Sci. USA 86:1173); and, self-sustained sequence replication (see, e.g., Guatelli (1990) Proc. Natl. Acad. Sci. USA 87:1874); Q Beta replicase amplification (see, e.g., Smith (1997) J. Clin. Microbiol. 35:1477-1491), automated Q-beta replicase amplification assay (see, e.g., Burg (1996) Mol. Cell. Probes 10:257-271) and other RNA polymerase mediated techniques (*e.g.*, NASBA, Cangene, Mississauga, Ontario); see also Berger (1987) Methods Enzymol. 152:307-316; Sambrook; Ausubel; U.S. Patent Nos. 4,683,195 and 4,683,202; Sooknanan (1995) Biotechnology 13:563-564.

Once amplified, the libraries can be cloned, if desired, into any of a variety of vectors using routine molecular biological methods; methods for cloning *in vitro* amplified nucleic acids are described, *e.g.*, U.S. Pat. No. 5,426,039. To facilitate cloning of amplified sequences, restriction enzyme sites can be "built into" the PCR primer pair.

The invention provides libraries of expression vectors encoding polypeptides and peptides of the invention. These nucleic acids may be introduced into a genome or into the cytoplasm or a nucleus of a cell and expressed by a variety of conventional techniques, well described in the scientific and patent literature. See, e.g., Roberts (1987) Nature 328:731; Schneider (1995) Protein Expr. Purif. 6435:10; Sambrook, Tijssen or Ausubel. The vectors can be isolated from natural sources, obtained from such sources as ATCC or GenBank libraries, or prepared by synthetic or recombinant methods. For example, the nucleic acids of the invention can be expressed in expression cassettes, vectors or viruses which are stably or transiently expressed in cells (e.g., episomal expression systems). Selection markers can be incorporated into expression cassettes and vectors to confer a selectable phenotype on transformed cells and sequences. For example, selection markers can code for episomal maintenance and replication such that integration into the host genome is not required.

In one embodiment, the nucleic acids of the invention are administered *in vivo* for *in situ* expression of the peptides or polypeptides of the invention. The nucleic acids can be administered as "naked DNA" (see, e.g., U.S. Patent No. 5,580,859) or in the form of an expression vector, e.g., a recombinant virus. The nucleic acids can be administered by any route, including peri- or intra-tumorally, as described below. Vectors administered *in vivo* can be derived from viral genomes, including recombinantly modified enveloped or non-enveloped DNA and RNA viruses, preferably selected from baculoviridiae, parvoviridiae, picornoviridiae, herpesveridiae, poxviridae, adenoviridiae, or picornnaviridiae. Chimeric vectors may also be employed which exploit advantageous merits of each of the parent vector properties (See e.g., Feng (1997) Nature Biotechnology 15:866-870). Such viral genomes may be modified by recombinant DNA techniques to include the nucleic acids of the invention; and may be further engineered to be replication deficient, conditionally replicating or replication competent. In alternative embodiments, vectors are derived from the adenoviral (e.g., replication incompetent vectors derived from the human adenovirus genome, see, e.g., U.S. Patent Nos. 6,096,718; 6,110,458; 6,113,913; 5,631,236); adeno-associated viral and retroviral genomes. Retroviral vectors can include those based upon murine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), Simian Immuno deficiency virus (SIV), human immuno deficiency virus (HIV), and combinations thereof;

see, e.g., U.S. Patent Nos. 6,117,681; 6,107,478; 5,658,775; 5,449,614; Buchscher (1992) J. Virol. 66:2731-2739; Johann (1992) J. Virol. 66:1635-1640). Adeno-associated virus (AAV)-based vectors can be used to transduce cells with target nucleic acids, e.g., in the *in vitro* production of nucleic acids and peptides, and in *in vivo* and *ex vivo* gene therapy procedures; see, e.g., U.S. Patent Nos. 6,110,456; 5,474,935; Okada (1996) Gene Ther. 3:957-964.

The peptides and polypeptides of the invention are derived from, or, based on, the structure of the kinase Cdc25C. The cDNA nucleic acid sequence for hCdc25C is

1 caggaagact ctgagtccga cgttggccta cccagtcgga aggcagagct gcaatctagt
61 taactacctc ctttccccta gatttccttt cattctgctc aagtcttcgc ctgtgtccga
121 tccctatcta ctttctctcc tcttgtagca agcctcagac tccaggcttg agctaggttt
181 tgtttttctc ctggtgagaa ttcgaagacc atgtctacgg aactcttctc atccacaaga
241 gaggaaggaa gctctggctc aggacccagt tttaggtcta atcaaaggaa aatgttaaac
301 ctgctcctgg agagagacac ttcctttacc gtctgtccag atgtccctag aactccagtg
361 ggcaaatttc ttggtgattc tgcaaaccta agcattttgt ctggaggaac cccaaaatgt
421 tgcctcgatc tttcgaatct tagcagtggg gagataactg ccactcagct taccacttct
481 gcagaccttg atgaaactgg tcacctggat tcttcaggac ttcaggaagt gcatttagct
541 gggatgaatc atgaccagca cctaatgaaa tgtagcccag cacagcttct ttgtagcact
601 ccgaatggtt tggaccgtgg ccatagaaag agagatgcaa tgtgtagttc atctgcaaat
661 aaagaaaatg acaatggaaa cttggtggac agtgaaatga aatatttggg cagtcccatt
721 actactgttc caaaattgga taaaaatcca aacctaggag aagaccaggc agaagagatt
781 tcagatgaat taatggagtt ttccctgaaa gatcaagaag caaaggtgag cagaagtggc
841 ctatatcgct ccccgtcgat gccagagaac ttgaacaggc caagactgaa gcaggtggaa
901 aaattcaagg acaacacaat accagataaa gttaaaaaaa agtatttttc tggccaagga
961 aagctcagga agggcttatg tttaaagaag acagtctctc tgtgtgacat tactatcact
1021 cagatgctgg aggaagattc taaccagggg cacctgattg gtgatttttc caaggtatgt
1081 gcgctgccaa ccgtgtcagg gaaacaccaa gatctgaagt atgtcaaccc agaaacagtg
1141 gctgccttac tgtcggggaa gttccagggt ctgattgaga agttttatgt cattgattgt
1201 cgctatccat atgagtatct gggaggacac atccagggag ccttaaactt atatagtcag
1261 gaagaactgt ttaacttctt tctgaagaag cccatcgtcc ctttggacac ccagaagaga
1321 ataatcatcg tgttccactg tgaattctcc tcagagaggg gcccccgaat gtgccgctgt
1381 ctgcgtgaag aggacaggtc tctgaaccag tatcctgcat tgtactaccc agagctatat
1441 atccttaaag gcggctacag agacttcttt ccagaatata tggaactgtg tgaaccacag
1501 agctactgcc ctatgcatca tcaggaccac aagactgagt tgctgaggtg tcgaagccag
1561 agcaaagtgc aggaagggga gcggcagctg cgggagcaga ttgcccttct ggtgaaggac

1621 atgagcccat gataacattc cagccactgg ctgctaacaa gtcaccaaaa agacactgca
1681 gaaaccctga gcagaaagag gccttctgga tggccaaacc caagattatt aaaagatgtc
1741 tctgcaaacc aacaggctac caacttgtat ccaggcctgg gaatggatta ggtttcagca
1801 gagctgaaag ctggtggcag agtcctggag ctggctctat aaggcagcct tgagttgcat
1861 agagatttgt attggttcag ggaactctgg cattcctttt cccaactcct catgtcttct
1921 cacaagccag ccaactcttt ctctctgggc ttcgggctat gcaagagcgt tgtctacctt
1981 ctttctttgt attttccttc tttgtttccc cctctttctt ttttaaaaat ggaaaaataa
2041 acactacaga atgag (SEQ ID NO:6)

The amino acid sequence of human hCdc25C is

MSTELFSSTREEGSSGSGPSFRSNQRKMLNLLLERDTSFTVCPD
VPRTPVGKFLGDSANLSILSGGTPKCCLDLSNLSSGEITATQLTTSADLDETGHLDSS
LQEVHLAGMNHDQHLMKCSPAQLLCSTPNGLDRGHRKRDAMCSSSANKENDNGNLVD
SEMKYLGSPITTVPKLDKNPNLGEDQAEEISDELMEFSLKDQEAKVSRSGLYRSPSMP
ENLNRPRLKQVEKFKDNTIPDKVKKKYFSGQGKLRKGLCLKKTVSLCDITITQMLEED
SNQGHLIGDFSKVCALPTVSGKHQDLKYVNPETVAALLSGKFQGLIEKFYVIDCRYPY
EYLGGHIQGALNLYSQEELFNFFLKKPIVPLDTQKRIIIVFHCEFSSERGPRMCRCLR
EEDRSLNQYPALYYPELYILKGGYRDFFPEYMELCEPQSYCPMHHQDHKTELLRCRSQ
SKVQEGERQLREQIALLVKDMSP (SEQ ID NO:1)

See also, e.g., GenBank Accession Nos. NP 001781 (protein) and NM 001790 (nucleic acid, cDNA) and Sadhu (1990) Proc. Natl. Acad. Sci. U.S.A. 87:5139-5143.

Peptides and Polypeptides

The peptides and polypeptides of the invention can be administered to treat cell proliferative disorders, including, e.g., to stop the growth of, or kill, cancer cells. The peptides and polypeptides of the invention can be used to inhibit (e.g., delay) or abrogate G2 cell cycle arrest checkpoint in cells. The peptides and polypeptides of the invention can also be used to inhibit Chk1 and/or Chk2/Cds1 kinase activity.

While the peptides and polypeptides of the invention can be expressed recombinantly *in vivo* after administration of nucleic acids, as described above, they can also be administered directly, e.g., as a pharmaceutical composition.

Polypeptides and peptides of the invention can be isolated from natural sources, be synthetic, or be recombinantly generated polypeptides. Peptides and proteins can be recombinantly expressed *in vitro* or *in vivo*. The peptides and polypeptides of the invention can be made and isolated using any method known in the art. Polypeptide and

peptides of the invention can also be synthesized, whole or in part, using chemical methods well known in the art. See e.g., Caruthers (1980) Nucleic Acids Res. Symp. Ser. 215-223; Horn (1980) Nucleic Acids Res. Symp. Ser. 225-232; Banga, A.K., Therapeutic Peptides and Proteins, Formulation, Processing and Delivery Systems (1995) Technomic Publishing Co., Lancaster, PA. For example, peptide synthesis can be performed using various solid-phase techniques (see e.g., Roberge (1995) Science 269:202; Merrifield (1997) Methods Enzymol. 289:3-13) and automated synthesis may be achieved, e.g., using the ABI 431A Peptide Synthesizer (Perkin Elmer) in accordance with the instructions provided by the manufacturer.

The peptides and polypeptides of the invention, as defined above, include all "mimetic" and "peptidomimetic" forms. The terms "mimetic" and "peptidomimetic" refer to a synthetic chemical compound which has substantially the same structural and/or functional characteristics of the polypeptides of the invention. The mimetic can be either entirely composed of synthetic, non-natural analogues of amino acids, or, is a chimeric molecule of partly natural peptide amino acids and partly non-natural analogs of amino acids. The mimetic can also incorporate any amount of natural amino acid conservative substitutions as long as such substitutions also do not substantially alter the mimetic's structure and/or activity. As with polypeptides of the invention which are conservative variants, routine experimentation will determine whether a mimetic is within the scope of the invention, i.e., that its structure and/or function is not substantially altered. Thus, a mimetic composition is within the scope of the invention if, when administered to or expressed in a cell, it disrupts the G2 cell cycle arrest checkpoint. A mimetic composition can also be within the scope of the invention if it can inhibit Chk1 and/or Chk2/Cds1 kinase activity, or, bind to the active site of either of these enzymes.

Polypeptide mimetic compositions can contain any combination of non-natural structural components, which are typically from three structural groups: a) residue linkage groups other than the natural amide bond ("peptide bond") linkages; b) non-natural residues in place of naturally occurring amino acid residues; or c) residues which induce secondary structural mimicry, i.e., to induce or stabilize a secondary structure, e.g., a beta turn, gamma turn, beta sheet, alpha helix conformation, and the like. For example, a polypeptide can be characterized as a mimetic when all or some of its residues are joined by chemical means other than natural peptide bonds. Individual peptidomimetic residues can be

joined by peptide bonds, other chemical bonds or coupling means, such as, e.g., glutaraldehyde, N-hydroxysuccinimide esters, bifunctional maleimides, N,N'-dicyclohexylcarbodiimide (DCC) or N,N'-diisopropylcarbodiimide (DIC). Linking groups that can be an alternative to the traditional amide bond ("peptide bond") linkages include, e.g., ketomethylene (e.g., -C(=O)-CH_2- for -C(=O)-NH-), aminomethylene (CH_2-NH), ethylene, olefin (CH=CH), ether (CH_2-O), thioether (CH_2-S), tetrazole (CN_4-), thiazole, retroamide, thioamide, or ester (see, e.g., Spatola (1983) in Chemistry and Biochemistry of Amino Acids, Peptides and Proteins, Vol. 7, pp 267-357, "Peptide Backbone Modifications," Marcell Dekker, NY).

A polypeptide can also be characterized as a mimetic by containing all or some non-natural residues in place of naturally occurring amino acid residues. Non-natural residues are well described in the scientific and patent literature; a few exemplary non-natural compositions useful as mimetics of natural amino acid residues and guidelines are described below. Mimetics of aromatic amino acids can be generated by replacing by, e.g., D- or L- naphylalanine; D- or L- phenylglycine; D- or L-2 thieneylalanine; D- or L-1, -2, 3-, or 4- pyreneylalanine; D- or L-3 thieneylalanine; D- or L-(2-pyridinyl)-alanine; D- or L-(3-pyridinyl)-alanine; D- or L-(2-pyrazinyl)-alanine; D- or L-(4-isopropyl)-phenylglycine; D-(trifluoromethyl)-phenylglycine; D-(trifluoromethyl)-phenylalanine; D-p-fluoro-phenylalanine; D- or L-p-biphenylphenylalanine; K- or L-p-methoxy-biphenylphenylalanine; D- or L-2-indole(alkyl)alanines; and, D- or L-alkylainines, where alkyl can be substituted or unsubstituted methyl, ethyl, propyl, hexyl, butyl, pentyl, isopropyl, iso-butyl, sec-isotyl, iso-pentyl, or a non-acidic amino acids. Aromatic rings of a non-natural amino acid include, e.g., thiazolyl, thiophenyl, pyrazolyl, benzimidazolyl, naphthyl, furanyl, pyrrolyl, and pyridyl aromatic rings.

Mimetics of acidic amino acids can be generated by substitution by, e.g., non-carboxylate amino acids while maintaining a negative charge; (phosphono)alanine; sulfated threonine. Carboxyl side groups (e.g., aspartyl or glutamyl) can also be selectively modified by reaction with carbodiimides (R'-N-C-N-R') such as, e.g., 1-cyclohexyl-3(2-morpholinyl-(4-ethyl) carbodiimide or 1-ethyl-3(4-azonia- 4,4- dimetholpentyl) carbodiimide. Aspartyl or glutamyl can also be converted to asparaginyl and glutaminyl residues by reaction with ammonium ions.

Mimetics of basic amino acids can be generated by substitution with, e.g., (in addition to lysine and arginine) the amino acids ornithine, citrulline, or (guanidino)-acetic acid, or (guanidino)alkyl-acetic acid, where alkyl is defined above. Nitrile derivative (e.g., containing the CN-moiety in place of COOH) can be substituted for asparagine or glutamine. Asparaginyl and glutaminyl residues can be deaminated to the corresponding aspartyl or glutamyl residues.

Arginine residue mimetics can be generated by reacting arginyl with, e.g., one or more conventional reagents, including, e.g., phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, or ninhydrin, preferably under alkaline conditions. Tyrosine residue mimetics can be generated by reacting tyrosyl with, e.g., aromatic diazonium compounds or tetranitromethane. N-acetylimidizol and tetranitromethane can be used to form O-acetyl tyrosyl species and 3-nitro derivatives, respectively. Cysteine residue mimetics can be generated by reacting cysteinyl residues with, e.g., alpha-haloacetates such as 2-chloroacetic acid or chloroacetamide and corresponding amines; to give carboxymethyl or carboxyamidomethyl derivatives. Cysteine residue mimetics can also be generated by reacting cysteinyl residues with, e.g., bromo-trifluoroacetone, alpha-bromo-beta-(5-imidozoyl) propionic acid; chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide; methyl 2-pyridyl disulfide; p-chloromercuribenzoate; 2-chloromercuri-4 nitrophenol; or, chloro-7-nitrobenzo-oxa-1,3-diazole. Lysine mimetics can be generated (and amino terminal residues can be altered) by reacting lysinyl with, e.g., succinic or other carboxylic acid anhydrides. Lysine and other alpha-amino-containing residue mimetics can also be generated by reaction with imidoesters, such as methyl picolinimidate, pyridoxal phosphate, pyridoxal, chloroborohydride, trinitrobenzenesulfonic acid, O-methylisourea, 2,4, pentanedione, and transamidase-catalyzed reactions with glyoxylate. Mimetics of methionine can be generated by reaction with, e.g., methionine sulfoxide. Mimetics of proline include, e.g., pipecolic acid, thiazolidine carboxylic acid, 3- or 4- hydroxy proline, dehydroproline, 3- or 4-methylproline, or 3,3,-dimethylproline. Histidine residue mimetics can be generated by reacting histidyl with, e.g., diethylprocarbonate or para-bromophenacyl bromide. Other mimetics include, e.g., those generated by hydroxylation of proline and lysine; phosphorylation of the hydroxyl groups of seryl or threonyl residues; methylation of the alpha-amino groups of lysine, arginine and histidine; acetylation of the N-terminal amine;

methylation of main chain amide residues or substitution with N-methyl amino acids; or amidation of C-terminal carboxyl groups.

A component of a polypeptide of the invention can also be replaced by an amino acid (or peptidomimetic residue) of the opposite chirality. Thus, any amino acid naturally occurring in the L-configuration (which can also be referred to as the R or S, depending upon the structure of the chemical entity) can be replaced with the amino acid of the same chemical structural type or a peptidomimetic, but of the opposite chirality, referred to as the D- amino acid, but which can additionally be referred to as the R- or S- form.

The skilled artisan will recognize that individual synthetic residues and polypeptides incorporating these mimetics can be synthesized using a variety of procedures and methodologies, which are well described in the scientific and patent literature, *e.g.*, Organic Syntheses Collective Volumes, Gilman, et al. (Eds) John Wiley & Sons, Inc., NY. Peptides and peptide mimetics of the invention can also be synthesized using combinatorial methodologies. Various techniques for generation of peptide and peptidomimetic libraries are well known, and include, *e.g.*, multipin, tea bag, and split-couple-mix techniques; see, *e.g.*, al-Obeidi (1998) Mol. Biotechnol. 9:205-223; Hruby (1997) Curr. Opin. Chem. Biol. 1:114-119; Ostergaard (1997) Mol. Divers. 3:17-27; Ostresh (1996) Methods Enzymol. 267:220-234. Modified peptides of the invention can be further produced by chemical modification methods, see, *e.g.*, Belousov (1997) Nucleic Acids Res. 25:3440-3444; Frenkel (1995) Free Radic. Biol. Med. 19:373-380; Blommers (1994) Biochemistry 33:7886-7896.

Peptides and polypeptides of the invention can also be synthesized and expressed as fusion proteins with one or more additional domains linked thereto for, *e.g.*, producing a more immunogenic peptide, to more readily isolate a recombinantly synthesized peptide, to identify and isolate antibodies and antibody-expressing B cells, and the like. Detection and purification facilitating domains include, *e.g.*, metal chelating peptides such as polyhistidine tracts and histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp, Seattle WA). The inclusion of a cleavable linker sequences such as Factor Xa or enterokinase (Invitrogen, San Diego CA) between a purification domain and the motif-comprising peptide or polypeptide to facilitate purification. For example, an expression vector can include an

epitope-encoding nucleic acid sequence linked to six histidine residues followed by a thioredoxin and an enterokinase cleavage site (see *e.g.*, Williams (1995) Biochemistry 34:1787-1797; Dobeli (1998) Protein Expr. Purif. 12:404-14). The histidine residues facilitate detection and purification while the enterokinase cleavage site provides a means for purifying the epitope from the remainder of the fusion protein. Technology pertaining to vectors encoding fusion proteins and application of fusion proteins are well described in the scientific and patent literature, see *e.g.*, Kroll (1993) DNA Cell. Biol., 12:441-53.

The invention provides methods for inhibiting a the activity of a Chk1 kinase or a Chk2 kinase. The invention also provides methods for screening for compositions that inhibit the activity of, or bind to (e.g., bind to the active site), Chk1 kinase and/or a Chk2 kinase. The amino acid sequence of human Chk1 kinase is

MAVPFVEDWDLVQTLGEGAYGEVQLAVNRVTEEAVAVKIVDMKR
AVDCPENIKKEICINKMLNHENVVKFYGHRREGNIQYLFLEYCSGGELFDRIEPDIGM
PEPDAQRFFHQLMAGVVYLHGIGITHRDIKPENLLLDERDNLKISDFGLATVFRYNNR
ERLLNKMCGTLPYVAPELLKRREFHAEPVDVWSCGIVLTAMLAGELPWDQPSDSCQEY
SDWKEKKTYLNPWKKIDSAPLALLHKILVENPSARITIPDIKKDRWYNKPLKKGAKRP
RVTSGGVSESPSGFSKHIQSNLDFSPVNSASSEENVKYSSSQPEPRTGLSLWDTSPSY
IDKLVQGISFSQPTCPDHMLLNSQLLGTPGSSQNPWQRLVKRMTRFFTKLDADKSYQC
LKETCEKLGYQWKKSCMNQVTISTTDRRNNKLIFKVNLLEMDDKILVDFRLSKGDGLE
FKRHFLKIKGKLIDIVSSQKVWLPAT (SEQ ID NO:3)

See also, Sanchez (1997) Science 277:1497-1501; Genbank Accession Nos. AF 016582; AAC 51736; NP 001265, NM 001274.

The amino acid sequence of human Chk2 kinasc is

MSRESDVEAQQSHGSSACSQPHGSVTQSQGSSSQSQGISSSSTS
MPNSSQSSHSSSGTLSSLETVSTQELYSIPEDQEPEDQEPEEPTPAPWARLWALQDG
FANLECVNDNYWFGRDKSCEYCFDEPLLKRTDKYRTYSKKHFRIFREVGPKNSYIAYI
EDHSGNGTFVNTELVGKGKRRPLNNNSEIALSLSRNKVFVFFDLTVDDQSVYPKALRD
EYIMSKTLGSGACGEVKLAFERKTCKKVAIKIISKRKFAIGSAREADPALNVETEIEI
LKKLNHPCIIKIKNFFDAEDYYIVLELMEGGELFDKVVGNKRLKEATCKLYFYQMLLA
VQYLHENGIIHRDLKPENVLLSSQEEDCLIKITDFGHSKILGETSLMRTLCGTPTYLA
PEVLVSVGTAGYNRAVDCWSLGVILFICLSGYPPFSEHRTQVSLKDQITSGKYNFIPE
VWAEVSEKALDLVKKLLVVDPKARFTTEEALRHPWLQDEDMKRKFQDLLSEENESTAL
PQVLAQPSTSRKRPREGEAEGAETTKRPAVCAAVL (SEQ ID NO:4)

See also Brown (1999) Proc. Natl. Acad. Sci. USA 96:3745-3750; Chaturvedi (1999) Oncogene 18:4047-4054; Genbank Accession Nos. NP 009125; NM 007194.

Antibody Generation

The invention provides antibodies that specifically bind to the peptides and polypeptides of the invention. These antibodies can be used to identify the presence of these peptides and polypeptides. The peptides and polypeptides of the invention can be used as immunogens to generate antibodies specific for a corresponding Cdc25C phosphatase. The anti-peptide antibodies of the invention can be used to generate anti-idiotype antibodies that specifically bind to active sites of Chk1 or Chk2 kinase.

Methods of producing polyclonal and monoclonal antibodies are known to those of skill in the art and described in the scientific and patent literature, see, *e.g.*, Coligan, CURRENT PROTOCOLS IN IMMUNOLOGY, Wiley/Greene, NY (1991); Stites (eds.) BASIC AND CLINICAL IMMUNOLOGY (7th ed.) Lange Medical Publications, Los Altos, CA ("Stites"); Goding, MONOCLONAL ANTIBODIES: PRINCIPLES AND PRACTICE (2d ed.) Academic Press, New York, NY (1986); Kohler (1975) Nature 256:495; Harlow (1988) ANTIBODIES, A LABORATORY MANUAL, Cold Spring Harbor Publications, New York. Antibodies can be generated *in vitro*, *e.g.*, using recombinant antibody binding site expressing phage display libraries, in addition to the traditional *in vivo* methods using animals. See, *e.g.*, Huse (1989) Science 246:1275; Ward (1989) Nature 341:544; Hoogenboom (1997) Trends Biotechnol. 15:62-70; Katz (1997) Annu. Rev. Biophys. Biomol. Struct. 26:27-45. Human antibodies can be generated in mice engineered to produce only human antibodies, as described by, e.g., U.S. Patent No. 5,877,397; 5,874,299; 5,789,650; and 5,939,598. B-cells from these mice can be immortalized using standard techniques (e.g., by fusing with an immortalizing cell line such as a myeloma or by manipulating such B-cells by other techniques to perpetuate a cell line) to produce a monoclonal human antibody-producing cell. See, e.g., U.S. Patent No. 5,916,771; 5,985,615. For making chimeric, *e.g.*, "humanized," antibodies, see *e.g.*, U.S. Patent Nos. 5,811,522; 5,789,554; 5,861,155. Alternatively, recombinant antibodies can also be expressed by transient or stable expression vectors in mammalian, including human, cells as in Norderhaug (1997) J. Immunol. Methods 204:77-87; Boder (1997) Nat. Biotechnol. 15:553-557; see also U.S. Patent No. 5,976,833

Screening for candidate compounds

The invention provides compositions and methods for screening for potential therapeutic compounds ("candidate compounds") to inhibit or abrogate Chk1 and/or Chk2/Cds1 kinase activity and/or the G2 cell cycle arrest checkpoint. For example, the screening can involve *in vitro* or *in vivo* assays wherein Chk1 and Chk2/Cds1 kinases phosphorylate peptides and polypeptides comprising the motifs of the invention; see Example 1, below. Inhibitors of peptide phosphorylation are candidate compounds. Alternatively, assays incorporating the experiments, or variations thereof, as set forth in Example 1, below, can be designed to assay for candidate compounds which can inhibit or abrogate Chk1 and/or Chk2/Cds1 kinase activity and/or the G2 cell cycle arrest checkpoint.

In one embodiment, the peptides and polypeptides of the invention can be bound to a solid support. Solid supports can include, e.g., membranes (e.g., nitrocellulose or nylon), a microtiter dish (e.g., PVC, polypropylene, or polystyrene), a test tube (glass or plastic), a dip stick (e.g., glass, PVC, polypropylene, polystyrene, latex and the like), a microfuge tube, or a glass, silica, plastic, metallic or polymer bead or other substrate such as paper. One solid support uses a metal (e.g., cobalt or nickel)-comprising column which binds with specificity to a histidine tag engineered onto a peptide.

Adhesion of peptides to a solid support can be direct (i.e. the protein contacts the solid support) or indirect (a particular compound or compounds are bound to the support and the target protein binds to this compound rather than the solid support). Peptides can be immobilized either covalently (e.g., utilizing single reactive thiol groups of cysteine residues (see, e.g., Colliuod (1993) Bioconjugate Chem. 4:528-536) or non-covalently but specifically (e.g., via immobilized antibodies (see, e.g., Schuhmann (1991) Adv. Mater. 3:388-391; Lu (1995) Anal. Chem. 67:83-87; the biotin/strepavidin system (see, e.g., Iwane (1997) Biophys. Biochem. Res. Comm. 230:76-80); metal chelating, e.g., Langmuir-Blodgett films (see, e.g., Ng (1995) Langmuir 11:4048-55); metal-chelating self-assembled monolayers (see, e.g., Sigal (1996) Anal. Chem. 68:490-497) for binding of polyhistidine fusions.

Indirect binding can be achieved using a variety of linkers which are commercially available. The reactive ends can be any of a variety of functionalities including, but not limited to: amino reacting ends such as N-hydroxysuccinimide (NHS) active esters, imidoesters, aldehydes, epoxides, sulfonyl halides, isocyanate, isothiocyanate,

and nitroaryl halides; and thiol reacting ends such as pyridyl disulfides, maleimides, thiophthalimides, and active halogens. The heterobifunctional crosslinking reagents have two different reactive ends, e.g., an amino-reactive end and a thiol-reactive end, while homobifunctional reagents have two similar reactive ends, e.g., bismaleimidohexane (BMH) which permits the cross-linking of sulfhydryl-containing compounds. The spacer can be of varying length and be aliphatic or aromatic. Examples of commercially available homobifunctional cross-linking reagents include, but are not limited to, the imidoesters such as dimethyl adipimidate dihydrochloride (DMA); dimethyl pimelimidate dihydrochloride (DMP); and dimethyl suberimidate dihydrochloride (DMS). Heterobifunctional reagents include commercially available active halogen-NHS active esters coupling agents such as N-succinimidyl bromoacetate and N-succinimidyl (4-iodoacetyl)aminobenzoate (SIAB) and the sulfosuccinimidyl derivatives such as sulfosuccinimidyl(4-iodoacetyl)aminobenzoate (sulfo-SIAB) (Pierce). Another group of coupling agents is the heterobifunctional and thiol cleavable agents such as N-succinimidyl 3-(2-pyridyidithio)propionate (SPDP) (Pierce Chemicals, Rockford, IL).

Antibodies can be used for binding polypeptides and peptides of the invention to a solid support. This can be done directly by binding peptide-specific antibodies to the column or it can be done by creating fusion protein chimeras comprising motif-containing peptides linked to, e.g., a known epitope (e.g., a tag (e.g., FLAG, myc) or an appropriate immunoglobulin constant domain sequence (an "immunoadhesin," see, e.g., Capon (1989) Nature 377:525-531 (1989).

There are a variety of assay formats that can be used to screen for "candidate compounds" to inhibit or abrogate Chk1 and/or Chk2/Cds1 kinase activity and/or the G2 cell cycle arrest checkpoint.. For example, as discussed above, compounds that inhibit the phosphorylation of the motif-comprising peptides of the invention can be candidate compounds. Alternatively, compounds that specifically bind to the motifs of the invention can be candidate compounds. For a general description of different formats for binding assays, see, e.g., BASIC AND CLINICAL IMMUNOLOGY, 7th Ed. (D. Stiles and A. Terr, ed.)(1991); ENZYME IMMUNOASSAY, E.T. Maggio, ed., CRC Press, Boca Raton, Florida (1980); and "Practice and Theory of Enzyme Immunoassays" in P. Tijssen, LABORATORY

TECHNIQUES IN BIOCHEMISTRY AND MOLECULAR BIOLOGY, Elsevier Science Publishers, B.V. Amsterdam (1985).

Combinatorial chemical libraries

Combinatorial chemical libraries are one means to assist in the generation of new chemical compound leads, i.e., compounds that inhibit Chk1 and/or Chk2/Cds1 kinase and/or inhibit or abrogate the G2 cell cycle arrest checkpoint. A combinatorial chemical library is a collection of diverse chemical compounds generated by either chemical synthesis or biological synthesis by combining a number of chemical "building blocks" such as reagents. For example, a linear combinatorial chemical library such as a polypeptide library is formed by combining a set of chemical building blocks called amino acids in every possible way for a given compound length (i.e., the number of amino acids in a polypeptide compound). Millions of chemical compounds can be synthesized through such combinatorial mixing of chemical building blocks. For example, the systematic, combinatorial mixing of 100 interchangeable chemical building blocks results in the theoretical synthesis of 100 million tetrameric compounds or 10 billion pentameric compounds (see, e.g., Gallop et al. (1994) 37(9): 1233-1250). Preparation and screening of combinatorial chemical libraries are well known to those of skill in the art, see, e.g., U.S. Patent No. 6,004,617; 5,985,356. Such combinatorial chemical libraries include, but are not limited to, peptide libraries (see, e.g., U.S. Patent No. 5,010,175; Furka (1991) Int. J. Pept. Prot. Res., 37: 487-493, Houghton et al. (1991) Nature, 354: 84-88). Other chemistries for generating chemical diversity libraries include, but are not limited to: peptoids (see, e.g., WO 91/19735), encoded peptides (see, e.g., WO 93/20242), random bio-oligomers (see, e.g., WO 92/00091), benzodiazepines (see, e.g., U.S. Patent No. 5,288,514), diversomers such as hydantoins, benzodiazepines and dipeptides (see, e.g., Hobbs (1993) Proc. Nat. Acad. Sci. USA 90: 6909-6913), vinylogous polypeptides (see, e.g., Hagihara (1992) J. Amer. Chem. Soc. 114: 6568), non-peptidal peptidomimetics with a Beta- D- Glucose scaffolding (see, e.g., Hirschmann (1992) J. Amer. Chem. Soc. 114: 9217-9218), analogous organic syntheses of small compound libraries (see, e.g., Chen (1994) J. Amer. Chem. Soc. 116: 2661), oligocarbamates (see, e.g., Cho (1993) Science 261:1303), and/or peptidyl phosphonates (see, e.g., Campbell (1994) J. Org. Chem. 59: 658). See also Gordon (1994) J. Med. Chem. 37:1385; for nucleic acid libraries, peptide nucleic acid libraries, see, e.g., U.S. Patent No. 5,539,083; for antibody libraries, see, e.g.,

Vaughn (1996) Nature Biotechnology 14:309-314; for carbohydrate libraries, see, e.g., Liang et al. (1996) Science 274: 1520-1522, U.S. Patent No. 5,593,853; for small organic molecule libraries, see, e.g., for isoprenoids U.S. Patent 5,569,588; for thiazolidinones and metathiazanones, U.S. Patent No. 5,549,974; for pyrrolidines, U.S. Patent Nos. 5,525,735 and 5,519,134; for morpholino compounds, U.S. Patent No. 5,506,337; for benzodiazepines U.S. Patent No. 5,288,514.

Devices for the preparation of combinatorial libraries are commercially available (see, e.g., U.S. Patent No. 6,045,755; 5,792,431 ; 357 MPS, 390 MPS, Advanced Chem Tech, Louisville KY, Symphony, Rainin, Woburn, MA, 433A Applied Biosystems, Foster City, CA, 9050 Plus, Millipore, Bedford, MA). A number of robotic systems have also been developed for solution phase chemistries. These systems include automated workstations, e.g., like the automated synthesis apparatus developed by Takeda Chemical Industries, LTD. (Osaka, Japan) and many robotic systems utilizing robotic arms (Zymate II, Zymark Corporation, Hopkinton, Mass.; Orca, Hewlett-Packard, Palo Alto, Calif.) which mimic the manual synthetic operations performed by a chemist. Any of the above devices are suitable for use with the present invention. The nature and implementation of modifications to these devices (if any) so that they can operate as discussed herein will be apparent to persons skilled in the relevant art. In addition, numerous combinatorial libraries are themselves commercially available (see, e.g., ComGenex, Princeton, N.J., Asinex, Moscow, Ru, Tripos, Inc., St. Louis, MO, ChemStar, Ltd, Moscow, RU, 3D Pharmaceuticals, Exton, PA, Martek Biosciences, Columbia, MD, etc.).

Formulation and Administration of Pharmaceutical Compositions

In one embodiment, the peptides and polypeptides of the invention are combined with a pharmaceutically acceptable carrier (excipient) to form a pharmacological composition. Pharmaceutically acceptable carriers can contain a physiologically acceptable compound that acts to, e.g., stabilize, or increase or decrease the absorption or clearance rates of the pharmaceutical compositions of the invention. Physiologically acceptable compounds can include, e.g., carbohydrates, such as glucose, sucrose, or dextrans, antioxidants, such as ascorbic acid or glutathione, chelating agents, low molecular weight proteins, compositions that reduce the clearance or hydrolysis of the peptides or polypeptides, or excipients or other stabilizers and/or buffers. Detergents can also used to stabilize or to increase or decrease the absorption of the pharmaceutical composition, including liposomal carriers. Pharmaceutically acceptable carriers and formulations for peptides and polypeptide are known to the skilled artisan and are described in detail in the scientific and patent literature, see e.g., the latest edition of Remington's Pharmaceutical Science, Mack Publishing Company, Easton, Pennsylvania ("Remington's").

Other physiologically acceptable compounds include wetting agents, emulsifying agents, dispersing agents or preservatives which are particularly useful for preventing the growth or action of microorganisms. Various preservatives are well known and include, e.g., phenol and ascorbic acid. One skilled in the art would appreciate that the choice of a pharmaceutically acceptable carrier including a physiologically acceptable compound depends, for example, on the route of administration of the peptide or polypeptide of the invention and on its particular physio-chemical characteristics.

In one embodiment, a solution of peptide or polypeptide of the invention is dissolved in a pharmaceutically acceptable carrier, e.g., an aqueous carrier if the composition is water-soluble. Examples of aqueous solutions that can be used in formulations for enteral, parenteral or transmucosal drug delivery include, e.g., water, saline, phosphate buffered saline, Hank's solution, Ringer's solution, dextrose/saline, glucose solutions and the like. The formulations can contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as buffering agents, tonicity adjusting agents, wetting agents, detergents and the like. Additives can also include additional active ingredients such as bactericidal agents, or stabilizers. For example, the solution can contain

sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate or triethanolamine oleate. These compositions can be sterilized by conventional, well-known sterilization techniques, or can be sterile filtered. The resulting aqueous solutions can be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile aqueous solution prior to administration. The concentration of peptide in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the patient's needs.

Solid formulations can be used for enteral (oral) administration. They can be formulated as, e.g., pills, tablets, powders or capsules. For solid compositions, conventional nontoxic solid carriers can be used which include, e.g., pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed, and generally 10% to 95% of active ingredient (e.g., peptide). A non-solid formulation can also be used for enteral administration. The carrier can be selected from various oils including those of petroleum, animal, vegetable or synthetic origin, e.g., peanut oil, soybean oil, mineral oil, sesame oil, and the like. Suitable pharmaceutical excipients include e.g., starch, cellulose, talc, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol.

Peptides and polypeptides of the invention, when administered orally, can be protected from digestion. This can be accomplished either by complexing the peptide or polypeptide with a composition to render it resistant to acidic and enzymatic hydrolysis or by packaging the peptide or complex in an appropriately resistant carrier such as a liposome. Means of protecting compounds from digestion are well known in the art, see, e.g., Fix (1996) Pharm Res. 13:1760-1764; Samanen (1996) J. Pharm. Pharmacol. 48:119-135; U.S. Patent 5,391,377, describing lipid compositions for oral delivery of therapeutic agents (liposomal delivery is discussed in further detail, infra).

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be

permeated can be used in the formulation. Such penetrants are generally known in the art, and include, e.g., for transmucosal administration, bile salts and fusidic acid derivatives. In addition, detergents can be used to facilitate permeation. Transmucosal administration can be through nasal sprays or using suppositories. See, e.g., Sayani (1996) "Systemic delivery of peptides and proteins across absorptive mucosae" Crit. Rev. Ther. Drug Carrier Syst. 13:85-184. For topical, transdermal administration, the agents are formulated into ointments, creams, salves, powders and gels. Transdermal delivery systems can also include, e.g., patches.

The peptides and polypeptide complexes can also be administered in sustained delivery or sustained release mechanisms, which can deliver the formulation internally. For example, biodegradeable microspheres or capsules or other biodegradeable polymer configurations capable of sustained delivery of a peptide can be included in the formulations of the invention (see, e.g., Putney (1998) Nat. Biotechnol. 16:153-157).

For inhalation, the peptide or polypeptide can be delivered using any system known in the art, including dry powder aerosols, liquids delivery systems, air jet nebulizers, propellant systems, and the like. See, e.g., Patton (1998) Biotechniques 16:141-143; product and inhalation delivery systems for polypeptide macromolecules by, e.g., Dura Pharmaceuticals (San Diego, CA) , Aradigm (Hayward, CA), Aerogen (Santa Clara, CA), Inhale Therapeutic Systems (San Carlos, CA), and the like. For example, the pharmaceutical formulation can be administered in the form of an aerosol or mist. For aerosol administration, the formulation can be supplied in finely divided form along with a surfactant and propellant. In another embodiment, the device for delivering the formulation to respiratory tissue is an inhaler in which the formulation vaporizes. Other liquid delivery systems include, e.g., air jet nebulizers.

In preparing pharmaceuticals of the present invention, a variety of formulation modifications can be used and manipulated to alter pharmacokinetics and biodistribution. A number of methods for altering pharmacokinetics and biodistribution are known to one of ordinary skill in the art. Examples of such methods include protection of the complexes in vesicles composed of substances such as proteins, lipids (for example, liposomes, see below), carbohydrates, or synthetic polymers (discussed above). For a general discussion of pharmacokinetics, see, e.g., Remington's, Chapters 37-39.

The peptide and polypeptide complexes used in the methods of the invention can be delivered alone or as pharmaceutical compositions by any means known in the art, e.g., systemically, regionally, or locally (e.g., directly into, or directed to, a tumor); by intraarterial, intrathecal (IT), intravenous (IV), parenteral, intra-pleural cavity, topical, oral, or local administration, as subcutaneous, intra-tracheal (e.g., by aerosol) or transmucosal (e.g., buccal, bladder, vaginal, uterine, rectal, nasal mucosa). Actual methods for preparing administrable compositions will be known or apparent to those skilled in the art and are described in detail in the scientific and patent literature, see e.g., Remington's. For a "regional effect," e.g., to focus on a specific organ, one mode of administration includes intra-arterial or intrathecal (IT) injections, e.g., to focus on a specific organ, e.g., brain and CNS (see e.g., Gurun (1997) Anesth Analg. 85:317-323). For example, intra-carotid artery injection if preferred where it is desired to deliver a peptide or polypeptide complex of the invention directly to the brain. Parenteral administration is a preferred route of delivery if a high systemic dosage is needed. Actual methods for preparing parenterally administrable compositions will be known or apparent to those skilled in the art and are described in detail, in e.g., Remington's,. See also, Bai (1997) J. Neuroimmunol. 80:65-75; Warren (1997) J. Neurol. Sci. 152:31-38; Tonegawa (1997) J. Exp. Med. 186:507-515.

In one embodiment, the pharmaceutical formulations comprising peptides or polypeptides of the invention are incorporated in lipid monolayers or bilayers, e.g., liposomes, see, e.g., U.S. Patent No. 6,110,490; 6,096,716; 5,283,185; 5,279,833. The invention also provides formulations in which water soluble peptides or complexes have been attached to the surface of the monolayer or bilayer. For example, peptides can be attached to hydrazide- PEG- (distearoylphosphatidyl) ethanolamine- containing liposomes (see, e.g., Zalipsky (1995) Bioconjug. Chem. 6:705-708). Liposomes or any form of lipid membrane, such as planar lipid membranes or the cell membrane of an intact cell, e.g., a red blood cell, can be used. Liposomal formulations can be by any means, including administration intravenously, transdermally (see, e.g., Vutla (1996) J. Pharm. Sci. 85:5-8), transmucosally, or orally. The invention also provides pharmaceutical preparations in which the peptides and/or complexes of the invention are incorporated within micelles and/or liposomes (see, e.g., Suntres (1994) J. Pharm. Pharmacol. 46:23-28; Woodle (1992) Pharm. Res. 9:260-265). Liposomes and liposomal formulations can be prepared according to standard methods and

are also well known in the art, see, e.g., Remington's; Akimaru (1995) Cytokines Mol. Ther. 1:197-210; Alving (1995) Immunol. Rev. 145:5-31; Szoka (1980) Ann. Rev. Biophys. Bioeng. 9:467, U.S. Pat. Nos. 4, 235,871, 4,501,728 and 4,837,028.

Treatment Regimens: Pharmacokinetics

The pharmaceutical compositions can be administered in a variety of unit dosage forms depending upon the method of administration. Dosages for typical peptide and polypeptide pharmaceutical compositions are well known to those of skill in the art. Such dosages are typically advisorial in nature and are adjusted depending on the particular therapeutic context, patient tolerance, etc. The amount of peptide or polypeptide adequate to accomplish this is defined as a "therapeutically effective dose." The dosage schedule and amounts effective for this use, i.e., the "dosing regimen," will depend upon a variety of factors, including the stage of the disease or condition, the severity of the disease or condition, the general state of the patient's health, the patient's physical status, age, pharmaceutical formulation and concentration of active agent, and the like. In calculating the dosage regimen for a patient, the mode of administration also is taken into consideration. The dosage regimen must also take into consideration the pharmacokinetics, i.e., the pharmaceutical composition's rate of absorption, bioavailability, metabolism, clearance, and the like. See, e.g., the latest Remington's; Egleton (1997) "Bioavailability and transport of peptides and peptide drugs into the brain" Peptides 18:1431-1439; Langer (1990) Science 249:1527-1533.

In therapeutic applications, compositions are administered to a patient suffering from a cancer in an amount sufficient to at least partially arrest the disease and/or its complications. For example, in one embodiment, a soluble peptide pharmaceutical composition dosage for intravenous (IV) administration would be about 0.01 mg/hr to about 1.0 mg/hr administered over several hours (typically 1, 3, or 6 hours), which can be repeated for weeks with intermittent cycles. Considerably higher dosages (e.g., ranging up to about 10 mg/ml) can be used, particularly when the drug is administered to a secluded site and not into the blood stream, such as into a body cavity or into a lumen of an organ, e.g., the cerebrospinal fluid (CSF).

EXAMPLES

The following examples are offered to illustrate, but not to limit the claimed invention.

Example 1: Administration of peptides of the invention to selectively sensitize cancer cells to DNA damaging agents

The invention provides compositions and methods for sensitizing cells, particularly cells with an impaired G1 cell cycle arrest checkpoint, such as cancer cells, to DNA damaging agents. The following example describes studies which demonstrate that the compositions and methods of the invention are effective for selectively killing cancer cells (versus normal cells, which have an unimpaired G1 checkpoint). Specifically, these experiments describes the synthesis and use of two exemplary polypeptides of the invention. Two peptides corresponding to amino acids 211 to 221 of human Cdc25C (SEQ ID NO:1) fused with a part of HIV-1-TAT (SEQ ID NO:5). These peptides were demonstrated to inhibit hChk1 kinase (SEQ ID NO:3) and Chk2/HuCds1 (SEQ ID NO:4) kinase activity *in vitro* and to specifically abrogate the G2 checkpoint *in vivo*.

Chemicals and reagents. Bleomycin and colchicine were purchased from Wako Pure Chemical Co. (Osaka, Japan). Hydroxyurea was purchased from Sigma Chemical Co. (St. Louis, MO). These chemicals were dissolved in distilled H_2O to 10, 5 and 50 mg/ml, respectively, and stored at 4°C. Antibodies against 14-3-3β were purchased from Santa Cruz Biotechnology (Santa Cruz, CA) and anti-rabbit IgG horseradish peroxidase-conjugated secondary antibodies were purchased from Amersham Life Sciences (Arlington Heights, IL). Antibodies against HA and c-myc, and protein G-Sepharose were purchased from Santa Cruz Biotechnology and Amersham Pharmacia Biotech (Uppsala, Sweden), respectively.

Cell culture and plasmids. A human T-cell leukemia-derived cell line, Jurkat, was cultured in RPMI 1640 (Sigma) supplemented with 10% fetal calf serum (IBL: Immuno-Biological Laboratories, Gunma, Japan) at 37°C/5% CO_2. Human pancreatic epitheloid carcinoma-derived cell lines, MIA PaCa2 and PANC1, were cultured in Eagle's MEM (IWAKI, Chiba, Japan) and Dulbecco's modified Eagle's medium with 4 mM l-glucose (Sigma) and 1.0 mM sodium pyruvate (Life Technologies, Inc., Grand Island, NY), respectively, and supplemented with 10% fetal calf serum at 37°C/5% CO_2. Normal human

peripheral blood lymphocytes were collected by Ficoll-Paque (Amersham Pharmacia, Biotech) density gradient. Two million cells/ml were cultured in RPMI 1640 supplemented with 10% fetal calf serum at 37°C/5% CO_2 in the presence of 5 μg/ml PHA (Life Technologies, Inc.) for a week. Baculovirus lysates that include HA-tagged hChk1 (SEQ ID NO:3) or c-myc-tagged Chk2/HuCds1 (SEQ ID NO:4) and plasmid for GST-Cdc25C (amino acid 200-256) were made as described in Matsuoka (1998) Science 282:1893-1897, and provided by Dr. Makoto Nakanishi (Department of Biochemistry, Nagoya City University.

Peptides. TAT-S216 peptide was synthesized so that it contained an NH_2-terminal 11 amino acid TAT protein transduction domain (YGRKKRRQRRR (SEQ ID NO:5); see, e.g., Nagahara (1998) Nature Med. 4:1449-1452) followed by a corresponding amino acid 211 to 221 derived from the human Cdc25C amino acid sequence (SEQ ID NO:1) (S216; LYRSPASMPENL). Serine-216 residue was changed to alanine in TAT-S216A (S216A; LYRSPSMPENL) (SEQ ID NO:6). The Cdc25C portion was partially deleted and substituted with glycine in TAT-Control (GGRSPAMPE) (SEQ ID NO:7). All peptides were synthesized by Sawady Technology Co. (Tokyo, Japan).

Purification of recombinant GST-Cdc25C proteins. *Escherichia coli* DH5α cells were transformed by GST-Cdc25C (200-256) plasmid. The cells were incubated with 0.1 mM isopropyl β-D-thiogalactoside for 2 hr, harvested, and lysed with a buffer containing 50 mM Tris HCl (pH8.0), 100 mM NaCl, 0.5% NP-40, 5 μg/ml aprotinin, 5 μg/ml pepstatin A and 5 μg/ml leupeptin. The lysate was sonicated, centrifuged for clarification and incubated with glutathione-Sepharose 4B™ beads for 1 hr at 4°C and washed five times.

Kinase assay. HA-tagged hChk1 (SEQ ID NO:3) and c-myc-tagged Chk2/HuCds1 (SEQ ID NO:4) expressed in insect cells using recombinant baculovirus (see, e.g., Kaneko (1999) Oncogene 18:3673-3681) were purified by immunoprecipitation using anti-HA or anti-c-myc antibodies and protein G-Sepharose. Immune complex kinase reaction was done in PBS with 1 mM DTT, 1 mM MgCl2 and 100 μCi of [γ-^{32}P] ATP (Amersham; 6000Ci/mmol) plus purified 1 μM GST-Cdc25C or 10 μM Cdc25C peptide (amino acid 211 to 221 of Cdc25C (SEQ ID NO:1); LYRSPSMPENL, Sawady Technology Co.) substrates at 30°C for 15 min in the presence of 10 μM TAT-S216, TAT-S216A or TAT-Control. After the reaction, samples were separated in 12% or 15% SDS-PAGE and autoradiographed to detect GST-Cdc25C or peptide phosphorylation.

Cell-cycle analysis. The cell cycle status of the cells treated with peptides and/or bleomycin or colchicine was analyzed by FACS, as described by Kawabe (1997) Nature 385:454-458. In brief, two million Jurkat cells were re-suspended and incubated in 300 µl Krishan's solution (0.1% Sodium citrate, 50 µg/ml PI, 20 µg/ml RNase A and 0.5% NP-40; see supra) for 1 hr at 4°C and analyzed by FACScan™ (Beckton Dickinson, Mountain View, CA) with the program CELLQuest™ (Beckton Dickinson).

Histone H1 kinase assay. Ten million Jurkat cells were treated with hydroxyurea (100 µg/ml), bleomycin (10 µg/ml), or colchicine (5 µg/ml) with or without addition of TAT-S216A, TAT-S216 or TAT-Control (10 µM) for 6 hr. The cells were washed in cold PBS and lysed at 4°C in 1 ml of buffer A (50 mM Tris pH 8, 2 mM DTT, 5 mM EDTA, 100 mMNaCl, 0.5% NP40, 20 mM Na_3V0_4, 50 mM NaF, 4 µM Okadaic acid, 5 µg/ml aprotinin, 5 µg/ml pepstatin A and 5 µg/ml leupeptin.). Twenty microliter of p13^{sucl} agarose beads (Upstate Biotechnology., Saranac, NY) were added to the cleared lysates, incubated for 4 hr at 4°C, and washed five times with buffer A without 5 mM EDTA, 20 mM Na_3V0_4, 50 mM NaF, 4 µM Okadaic acid. Histone H1 kinase activity on the beads were analyzed by using Cdc2 kinase assay kit (Upstate Biotechnology) with [γ -^{32}P] ATP followed by 12% SDS-PAGE electrophoresis, and autoradiographed to detect the phosphorylated Histone H1.

Cell cytotoxicity assay. MIA PaCa2 and PANC1 cells (3x10^3/well) were plated in 96-well microtiter plates. After an overnight adherence, cells were treated with bleomycin (10 µg/ml) with or without the indicated TAT-peptides at various time points up to 96 hr. Cytotoxicity and cell survival were determined by the 3'-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis (4-methoxy-6-nitro) benzene sulfonic acid hydrate) (XTT) assay (Cell Proliferation Kit II™: Boehringer Mannheim, Germany), which was done according to company's protocol and Scudiero (1988) Cancer Res. 48.4827-4833.

TAT-S216 and TAT-S216A peptides inhibit hChk1 and Chk2/HuCds1 kinase activities

To inhibit hChk1 (SEQ ID NO:3) and Chk2/HuCds1 (SEQ ID NO:4) kinase activities and to abrogate DNA damage-induced-G2 arrest, synthetic peptides comprising amino acid residues 211 to 221 of Cdc25C (SEQ ID NO:1) and a variation of the TAT protein transduction domain (YGRKKRRQRRR (SEQ ID NO:5) (TAT-S216) were generated.

The results are shown in Figure 1: TAT-S216A and TAT-S216 peptides inhibit hChk1 and Chk2/HuCds1 kinase activities *in vitro*. Figure 1A. sequences of the peptides. Figure 1B, in vitro phosphorylation analysis using GST-Cdc25C and purified hChk1. GST-Cdc25C (amino acid 200-256) that was produced in E. coli (DH5α) was used as substrate (1 μM). Immune complex kinase reaction was done in the presence of TAT-S216A (10 μM) or TAT-S216 (10 μM). Figure 1C, in vitro phosphorylation analysis of hChk1 and Chk2/HuCds1 using synthesized Cdc25C peptide corresponding amino acid 211-221 of Cdc25C (LYRSPSMPENL) as a substrate (10 μM).

A TAT-S216A peptide (S216A; LYRSPSMPENL, (SEQ ID NO:6)), in which serine residue 216 was substituted by alanine was devised to stabilize the transient status of its interaction with hChk1 (SEQ ID NO:3) and Chk2/HuCds1 (SEQ ID NO:4) (Fig. 1A). This TAT peptide was included to efficiently transduce these peptides into cells (see, e.g., Nagahara (1998) supra). This sequence is known to facilitate the uptake of heterologous proteins across the cell membrane. As a control peptide, part of the Cdc25C portion of this peptide was deleted (TAT-Control).

As shown in Fig. 1B, hChk1 (SEQ ID NO:3) was capable of phosphorylating a Cdc25C protein (residues 200-256) (SEQ ID NO:1) fused to GST. Serine-216 on Cdc25C (SEQ ID NO:1) is the major phosphorylation site of this fusion protein *in vivo* (see, e.g., Furnari (1997) Science 277:1495-1497; Sanchez (1997) Science 277:1497-1501; Peng (1997) Science 277:1501-1505).

In Fig. 1B, both TAT-S216 and TAT-S216A inhibited the phosphorylation of Cdc25C by baculovirus-produced hChk1 (SEQ ID NO:3). TAT-S216 but not TAT-S216A was efficiently phosphorylated by hChk1, suggesting that serine-216 on TAT-S216 was phosphorylated by hChk1 and TAT-S216 would competitively inhibit substrate

phosphorylation at excess molar ratio if present in great enough quantity. TAT-Control peptide did not inhibit hChk1 kinase activity.

As shown in Fig. 1C, TAT-S216A significantly inhibited phosphorylation of Cdc25C peptide (residues 200-256) (SEQ ID NO:1) mediated by hChk1 (SEQ ID NO:3) and Chk2/HuCds1 (SEQ ID NO:4) even at a low stoichiometry (at four times more molar excess of TAT-S216A peptide against substrate Cdc25C peptide).

Abrogation of DNA damage-induced G2 checkpoint by TAT-S216 and TAT-S216A peptides

The cell cycle status of the cells treated with TAT-S216A or TAT-S216 upon the DNA damage-induced G2 arrest was analyzed by FACS analysis. Histone H1 kinase activities of theses cells were simultaneously monitored. Jurkat cells arrested exclusively at G2 by bleomycin (10 μg/ml) treatment, because it does not have functional p53. Results are shown in Figure 2: abrogation of DNA damage-induced G2 arrest by TAT-S216A and TAT-S216 peptides. Figure 2A, FACS analysis of Jurkat cells treated with bleomycin and peptides. Cells were treated with bleomycin (10 μg/ml) with or without peptides (10 μM) for 20 hr. B, histone H1 kinase analysis. Cell lysates were prepared from the cells treated with the indicated reagent for 6 hr. Concentrations used were: hydroxyurea (HU), 100 μg/ml; bleomycin (Bleo), 10 μg/ml; colchicine, 5 μg/ml; TAT-S216A and TAT-S216, 10 μM. C, FACS analysis of colchicine -and peptide-treated cells. Jurkat cells were treated with colchicine (5 μg/ml) with or without peptide (10 μM) for 20 hr.

As shown in Fig. 2A, G2 arrest was completely abrogated by the addition of TAT-S216A or TAT-S216 in response to bleomycin. G2 arrest was abrogated at any time point between 12 and 48 hr by the treatment with TAT-S216A or TAT-S216. Jurkat cells treated with bleomycin together with TAT-Control arrested at G2 similarly to the cells treated with bleomycin alone.

We also observed that either TAT-S216A or TAT-S216 also abrogated G2 arrest induced by gamma-irradiation and cisplatin (gamma-irradiation, 5 Gy; cisplatin, 1 μg/ml for 1 hr treatment). To further analyze the effect of these peptides on G2/M transition, histone H1 kinase activity was monitored. Consistent with the above findings, although histone H1 kinase activity was decreased by the treatment with bleomycin or hydroxyurea, it was unchanged or rather increased by the treatment with bleomycin in the presence of TAT-

S216A or TAT-S216 (Fig. 2B). In the presence of TAT-Control peptide, the bleomycin treatment did not affect with H1 kinase activity.

As shown in Fig. 2C, The M-phase arrest of Jurkat cells induced by colchicine was not affected by the addition of TAT-S216 or TAT-S216A. These results demonstrate that TAT-S216A and TAT-S216 specifically abrogated the DNA damage-activated cell cycle G2 checkpoint by inhibiting hChk1 (SEQ ID NO:3) and/or Chk2/HuCds1 (SEQ ID NO:4) kinase activities.

Sensitization of Jurkat cells to the bleomycin-induced cell death by TAT-S216A and TAT-S216 peptides

The effect of TAT-S216A and TAT-S216 on the cell death induced by bleomycin was examined. The results are shown in Figure 3; Trypan blue dye exclusion analysis of Jurkat cells treated with bleomycin (A) or colchicine (B) with or without indicated peptides. Bars, SD Vertical axis, % viability of the cells; Bleo 5, bleomycin 5 μg/ml; Bleo 10, bleomycin 10 μg/ml; colchicine, 5 μg/ml; TAT-S216 or TAT-S216A, 10 μM of indicated peptide. Note that TAT-S216A and TAT-S216 peptides did not increase the cytotoxicity of bleomycin to normal cells. C, survival analysis of PHA blasts treated with bleomycin and peptides. Vertical axis, % viability of the cells determined by trypan blue dye exclusion analysis; horizontal axis, time in hours. Bleo 5, bleomycin 5 μg/ml; Bleo 10, bleomycin 10 μg/ml; TAT-S216 or TAT-S216A, 10 μM of indicated peptide. D, FACS analysis of the cells treated with bleomycin and peptides. PHA-blasts were treated with bleomycin with or without peptides for 20 hr. Vertical axis, cell number; horizontal axis, DNA content indicated by propidium iodide staining.

As shown in Fig. 3A, the addition of TAT-S216A and TAT-S216 efficiently sensitized Jurkat cells to the bleomycin-induced cell death. Whereas bleomycin treatment at 5 or μ10 g/ml killed Jurkat cells by only 27-30%, the addition of 10 μM TAT-216A or TAT-S216 killed Jurkat cells by nearly 80%. In contrast, these peptide by themselves did not show any significant cytotoxicity. In addition, a control peptide TAT-Control did not affect the viability of bleomycin-treated Jurkat cells. Moreover, as expected from the result in Fig. 2C, either TAT-S216A or TAT-S216 did not affect the cytotoxicity by colchicine (Fig. 3B). This observation indicates that thc cell death induced by these peptides in the presence of bleomycin was not attributable to a nonspecific cytotoxic effect.

TAT-S216 and TAT-S216A peptides did not affect the viability of normal cells.

In order to confirm the specificity of the effect of these peptides on cancer cells in which the G1 checkpoint is abrogated, the effect of these peptides on normal human cells was investigated. Mitogen-activated normal human T lymphocytes (PHA blasts) were prepared by stimulating peripheral blood mononuclear cells obtained from a healthy donor with PHA for 1 week. These cells were treated with bleomycin (5 and 10 μ g/ml) in the presence or absence of either TAT-S216A or TAT-S216.

As shown in Fig. 3C, these peptides did not augment the cytotoxic effect of bleomycin, although these cells replicated as fast as Jurkat cells. As shown in Fig. 3D, PHA blasts treated with bleomycin (5 μ g/ml) arrested at G1 and S phase but not G2, presumably because of the activity of wild-type p53. When these cells were treated with TAT-S216 or TAT-S216A in addition to bleomycin, no further alteration of cell cycle pattern was observed.

Sensitization of pancreatic cancer cells to the bleomycin-induced cell death by TAT-S216A and TAT-S216 peptides

The effect of these peptides on two other p53-defective pancreatic cancer cell lines, MIA PaCa2 and PANC1 cells, was examined. Figure 4 shows the results of survival analysis of PANC1 (A) and MIA PaCa2 (B) cells treated with bleomycin and peptides. PANC1 and MIA PaCa2 cells were treated with bleomycin with or without the indicated peptide. The cell viability was determined by the 3'-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis (4-methoxy-6-nitro) benzene sulfonic acid hydrate assay at the indicated times after addition of bleomycin and peptide. Bleo 60, bleomycin 60 μg/ml; TAT-S216 or TAT-S216A, 10 μM of indicated peptide. Bars, SD.

Although these pancreatic cancer cells are known to be resistant to various anti-cancer reagents, these cells could also be sensitized to the bleomycin-induced cell death by TAT-S216A and TAT-S216 (Fig. 4). Similarly, these peptides could sensitize these cells to the cell death induced by other DNA-damaging agents including cisplatin and gamma-irradiation.

In summary, these experiments demonstrated for the first time that short peptides that inhibit both hChk1 and Chk2/HuCds1 kinase activities can specifically abrogate the DNA damage-induced G2 cell growth arrest checkpoint. These data also demonstrated

that the specific abrogation of the G2 checkpoint sensitized cancer cells to bleomycin, DNA-damaging agent, without obvious effect on normal cell cycle and its viability. These observations indicate that these kinases involved in G2 cell cycle checkpoint are ideal targets for the specific abrogation of G2 checkpoint and that the peptides and polypeptides of the invention and their derivatives can be used in novel cancer therapy.

Example 2: Optimization of sequences for G2 abrogating peptides of the invention

The following example describes studies which identified exemplary G2 checkpoint-abrogating peptides of the invention. This was accomplished by using a computer analysis of the structure of human Chk2 kinase (SEQ ID NO:4) and the peptides of the invention.

The 3-dimensional structure of human Chk2 was predicted by comparing the primary and 3-D structure of another serine threonine kinase, PKA (PDB protein data base, Research Collaboratory for Structural Bioinformatics (RCSB), The National Science Foundation, Arlington, VA) (1CDK), using a computer program, MODELER™ (IMMD, Tokyo, Japan). The alignment of the peptides of the invention and hChk2 were predicted by comparing an alignment of hChk1 and various Cdc25C peptides as described by Chen (2000) "The 1.7 A crystal structure of human cell cycle checkpoint kinase Chk1: implications for Chk1 regulation," Cell 100:681-92. By comparing the predicted structure of hChk2 with the peptides of the invention, it was predicted that there are four pockets on hChk2 that are important for the interaction with peptides, as shown in Figure 5, P1, P2, P3 and P4. The structure of these pockets was used to design and confirm the sequences of exemplary peptides of the invention

The ability of these peptides to abrogate the activity of Chk2 kinase, thereby imbuing the ability to abrogate the G2 cell cycle checkpoint, was demonstrated by their ability to act as a phosphorylation substrate for human Chk2 kinase. Exemplary peptides were directly synthesized (immobilized) on a membrane and contacted with human Chk2 kinase. Specifically, oligo-peptides with all sequences predicted by the 3-dimensional model were directly synthesized on a membrane by using an auto-spot-peptide-synthesizer, Model ASP-22 2 (ABiMED, Germany). The amount of peptide was about 0.1 micro-mol/cm^2.

The membrane was incubated with 2% Gly-Gly in PBS for 2 hours (hr) at room temperature (RT). Then, they were washed three times with 0.1% Tween-P BS™. The

"kination," or "phosphorylation," reaction was performed with a recombinant fusion protein Gst-Chk2 at a concentration of about 5 μg in 4 ml reaction buffer, 1 mM $MgCl_2$, 2% Gly-Gly and γ-^{33}P-ATP in PBS at RT for 1 hr. After the reaction, the membrane was washed 5 times with RIPA (1% SDS, 1% NP-40, 100 mM NaCl) and analyzed with a Bass 2500™ image analyzer (Fuji, Japan). The signal was graded to "-," a "+," a "++," or a "+++." Table 1 shows the peptide sequences that gave signals stronger than "++." The peptides RYSLPPELSNM and LYRSPSAMPENL gave "+" signals by this analysis.

All of the following peptides were phosphorylated by human Chk2 kinase; in position "X" (corresponding to position X_8), wherein X = P, F, Y, or W, the signal was strongest (a "+++") when X = the amino acid tyrosine (Y):

37-40 L Y R S P S H X E N L
52-53 L Y S S P S Y X E N L
92-95 L Y T S P S Y X E N L
117-121 L Y T S P S H X E N L
132-135 L Y H S P S Y X E N L
1127-1130 W Y R S P S F X E N L
1237-1240 W Y T S P S H X E N L
372-375 L F T S P S Y X E N L
637-640 F Y S S P S H X E N L
642-645 F Y T S P S M X E N L
648-651 F Y T S P S F X E N L
652-655 F Y T S P S Y X E N L
1202-1205 W Y T S P S M X E N L
1207-1210 W Y T S P S F X E N L
1212-1215 W Y T S P S Y X E N L

The best phosphorylation substrates were the peptides L Y R S P S Y Y E N L and W Y T S P S Y F E N L.

The following Table 1 is a complete list of tested peptides and results of the *in vitro* phosphorylation by human Chk2 kinase assay. Results are presented to the right of the peptide, below: a "+++" indicates the peptide was relatively highly phosphorylated; a "++" indicates the peptide was relatively less phosphorylated, a "+" indicates the peptide was

detectably significantly phosphorylated over negative control, and no indication indicates that a peptide was not significantly phosphorylated over negative control (note: the number immediately to the right of the peptide is the MW of the peptide).

Table 1

1 R Y S L P P E L S N M 1308.6	+
2 L Y R S P S M P E N L 1308.6	+
3 L Y R S P S M F E N L 1358.6	-
4 L Y R S P S M Y E N L 1374.6	-
5 L Y R S P S M W E N L 1397.7	-
7 L Y R S P S F P E N L 1324.5	-
8 L Y R S P S F F E N L 1374.5	-
9 L Y R S P S F Y E N L 1390.5	-
10 L Y R S P S F W E N L 1413.6	-
12 L Y R S P S Y P E N L 1340.5	+
13 L Y R S P S Y F E N L 1390.5	+
14 L Y R S P S Y Y E N L 1406.5	+
15 L Y R S P S Y W E N L 1429.6	+
17 L Y R S P S D P E N L 1292.4	-
18 L Y R S P S D F E N L 1342.4	-
19 L Y R S P S D Y E N L 1358.4	-
20 L Y R S P S D W E N L 1381.5	-
22 L Y R S P S E P E N L 1306.4	-
23 L Y R S P S E F E N L 1356.4	-
24 L Y R S P S E Y E N L 1372.4	-
25 L Y R S P S E W E N L 1395.5	-
27 L Y R S P S N P E N L 1291.5	+
28 L Y R S P S N F E N L 1341.5	+
29 L Y R S P S N Y E N L 1357.5	+
30 L Y R S P S N W E N L 1380.6	+
32 L Y R S P S Q P E N L 1305.5	-
33 L Y R S P S Q F E N L 1355.5	-
34 L Y R S P S Q Y E N L 1371.5	-
35 L Y R S P S Q W E N L 1394.6	-
37 L Y R S P S H P E N L 1314.5	+

1 R Y S L P P E L S N M 1308.6
2 L Y R S P S M P E N L 1308.6

38	LYRSPSHFENL	1364.5	+
39	LYRSPSHYENL	1380.5	+
40	LYRSPSHWENL	1403.6	+
42	LYSSPSMPENL	1240.3	+
43	LYSSPSMFENL	1290.3	+
44	LYSSPSMYENL	1306.3	+
45	LYSSPSMWENL	1329.4	+
47	LYSSPSFPENL	1256.2	+
48	LYSSPSFFENL	1306.2	+
49	LYSSPSFYENL	1322.2	+
50	LYSSPSFWENL	1345.3	+
52	LYSSPSYPENL	1272.2	+
53	LYSSPSYFENL	1322.2	+
54	LYSSPSYYENL	1338.2	+
55	LYSSPSYWENL	1361.3	+
57	LYSSPSDPENL	1224.1	-
58	LYSSPSDFENL	1274.1	-
59	LYSSPSDYENL	1290.1	-
60	LYSSPSDWENL	1313.2	-
62	LYSSPSEPENL	1238.1	-
63	LYSSPSEFENL	1288.1	-
64	LYSSPSEYENL	1304.1	-
65	LYSSPSEWENL	1327.2	-
67	LYSSPSNPENL	1223.2	-
68	LYSSPSNFENL	1273.2	-
69	LYSSPSNYENL	1289.2	-
70	LYSSPSNWENL	1312.3	-
72	LYSSPSQPENL	1237.2	+
73	LYSSPSQFENL	1287.2	-
74	LYSSPSQYENL	1303.2	-
75	LYSSPSQWENL	1326.3	+
77	LYSSPSHPENL	1246.2	++
78	LYSSPSHFENL	1296.2	++
79	LYSSPSHYENL	1312.2	++
80	LYSSPSHWENL	1335.3	++
82	LYTSPSMPENL	1253.5	++

37	LYRSPSHPENL	1314.5
38	LYRSPSHFENL	1364.5
39	LYRSPSHYENL	1380.5
40	LYRSPSHWENL	1403.6
52	LYSSPSYPENL	1272.2
53	LYSSPSYFENL	1322.2
54	LYSSPSYYENL	1338.2
55	LYSSPSYWENL	1361.3
72	LYSSPSQPENL	1237.2
75	LYSSPSQWENL	1326.3
92	LYTSPSYPENL	1285.4
93	LYTSPSYFENL	1335.4
94	LYTSPSYYENL	1351.4
95	LYTSPSYWENL	1374.5
117	LYTSPSHPENL	1259.4
118	LYTSPSHFENL	1309.4
119	LYTSPSHYENL	1325.4
120	LYTSPSHWENL	1348.5
132	LYHSPSYPENL	1321.5
133	LYHSPSYFENL	1371.5
134	LYHSPSYYENL	1387.5
135	LYHSPSYWENL	1410.6
1127	WYRSPSFPENL	1397.6
1128	WYRSPSFFENL	1447.6
1129	WYRSPSFYENL	1463.
1130	WYRSPSFWENL	1486.
1237	WYTSPSHPENL	1332.
1238	WYTSPSHFENL	1382.
1239	WYTSPSHYENL	1398.

83	L Y T S P S M F E N L 1303.5	++
84	L Y T S P S M Y E N L 1319.5	++
85	L Y T S P S M W E N L 1342.6	++
87	L Y T S P S F P E N L 1269.4	++
88	L Y T S P S F F E N L 1319.4	++
89	L Y T S P S F Y E N L 1335.4	++
90	L Y T S P S F W E N L 1358.5	++
92	L Y T S P S Y P E N L 1285.4	++
93	L Y T S P S Y F E N L 1335.4	++
94	L Y T S P S Y Y E N L 1351.4	+++
95	L Y T S P S Y W E N L 1374.5	++
97	L Y T S P S D P E N L 1237.3	-
98	L Y T S P S D F E N L 1287.3	-
99	L Y T S P S D Y E N L 1303.3	-
100	L Y T S P S D W E N L 1326.4	-
102	L Y T S P S E P E N L 1251.3	-
103	L Y T S P S E F E N L 1301.3	-
104	L Y T S P S E Y E N L 1317.3	-
105	L Y T S P S E W E N L 1340.4	-
107	L Y T S P S N P E N L 1236.4	+
108	L Y T S P S N F E N L 1286.4	+
109	L Y T S P S N Y E N L 1302.4	+
110	L Y T S P S N W E N L 1325.5	+
112	L Y T S P S Q P E N L 1250.4	-
113	L Y T S P S Q F E N L 1300.4	-
114	L Y T S P S Q Y E N L 1316.4	-
115	L Y T S P S Q W E N L 1339.5	-
117	L Y T S P S H P E N L 1259.4	+
118	L Y T S P S H F E N L 1309.4	+
119	L Y T S P S H Y E N L 1325.4	+
120	L Y T S P S H W E N L 1348.5	+
122	L Y H S P S M P E N L 1289.6	-
123	L Y H S P S M F E N L 1339.6	-
124	L Y H S P S M Y E N L 1355.6	-
125	L Y H S P S M W E N L 1378.7	-
127	L Y H S P S F P E N L 1305.5	-

1240	W Y T S P S H W E N L 1421
372	L F T S P S Y P E N L 1269.4
373	L F T S P S Y F E N L 1319.4
374	L F T S P S Y Y E N L 1335.4
375	L F T S P S Y W E N L 1358.5
637	F Y S S P S H P E N L 1280.2
638	F Y S S P S H F E N L 1330.2
639	F Y S S P S H Y E N L 1346.2
640	F Y S S P S H W E N L 1369.3
642	F Y T S P S M P E N L 1287.5
643	F Y T S P S M F E N L 1337.5
644	F Y T S P S M Y E N L 1353.5
645	F Y T S P S M W E N L 1376.
647	F Y T S P S F P E N L 1303.4
648	F Y T S P S F F E N L 1353.4
649	F Y T S P S F Y E N L 1369.4
650	F Y T S P S F W E N L 1392.5
652	F Y T S P S Y P E N L 1319.4
653	F Y T S P S Y F E N L 1369.4
654	F Y T S P S Y Y E N L 1385.4
655	F Y T S P S Y W E N L 1408.5
1202	W Y T S P S M P E N L 1326.
1203	W Y T S P S M F E N L 1376.
1204	W Y T S P S M Y E N L 1392
1205	W Y T S P S M W E N L 141
1207	W Y T S P S F P E N L 1342.
1208	W Y T S P S F F E N L 1392.
1209	W Y T S P S F Y E N L 1408.
1210	W Y T S P S F W E N L 1431.
1212	W Y T S P S Y P E N L 1358.
1213	W Y T S P S Y F E N L 1408.
1214	W Y T S P S Y Y E N L 1424.
1215	W Y T S P S Y W E N L 1447
1	I R Y S L P P E L S N M 1308.6
2	L Y R S P S M P E N L 1308.6

128 L Y H S P S F F E N L 1355.5	-	2274 L K R S P S M P E N L	
129 L Y H S P S F Y E N L 1371.5	-	2342 L Y R S P S M V E N L	
130 L Y H S P S F W E N L 1394.6	-	2292 L Y I S P S M P E N L	
132 L Y H S P S Y P E N L 1321.5	+	2254 K Y R S P S M P E N L	
133 L Y H S P S Y F E N L 1371.5	+		
134 L Y H S P S Y Y E N L 1387.5	+++		
135 L Y H S P S Y W E N L 1410.6	+		
137 L Y H S P S D P E N L 1273.4	-		
138 L Y H S P S D F E N L 1323.4	-		
139 L Y H S P S D Y E N L 1339.4	-		
140 L Y H S P S D W E N L 1362.5	-		
142 L Y H S P S E P E N L 1287.4	-		
143 L Y H S P S E F E N L 1337.4	-		
144 L Y H S P S E Y E N L 1353.4	-		
145 L Y H S P S E W E N L 1376.5	-		
147 L Y H S P S N P E N L 1272.5	-		
148 L Y H S P S N F E N L 1322.5	-		
149 L Y H S P S N Y E N L 1338.5	-		
150 L Y H S P S N W E N L 1361.6	-		
152 L Y H S P S Q P E N L 1286.5	-		
153 L Y H S P S Q F E N L 1336.5	-		
154 L Y H S P S Q Y E N L 1352.5	-		
155 L Y H S P S Q W E N L 1375.6	-		
157 L Y H S P S H P E N L 1295.5	-		
158 L Y H S P S H F E N L 1345.5	-		
159 L Y H S P S H Y E N L 1361.5	-		
160 L Y H S P S H W E N L 1384.6	-		
162 L Y N S P S M P E N L 1266.6	-		
163 L Y N S P S M F E N L 1316.6	-		
164 L Y N S P S M Y E N L 1332.6	-		
165 L Y N S P S M W E N L 1355.7	-		
167 L Y N S P S F P E N L 1282.5	-		
168 L Y N S P S F F E N L 1332.5	-		
169 L Y N S P S F Y E N L 1348.5	-		
170 L Y N S P S F W E N L 1371.6	-		
172 L Y N S P S Y P E N L 1298.5	-		

173	L Y N S P S Y F E N L	1348.5	-
174	L Y N S P S Y Y E N L	1364.5	-
175	L Y N S P S Y W E N L	1387.6	-
177	L Y N S P S D P E N L	1250.4	-
178	L Y N S P S D F E N L	1300.4	-
179	L Y N S P S D Y E N L	1316.4	-
180	L Y N S P S D W E N L	1339.5	-
182	L Y N S P S E P E N L	1264.4	-
183	L Y N S P S E F E N L	1314.4	-
184	L Y N S P S E Y E N L	1330.4	-
185	L Y N S P S E W E N L	1353.5	-
187	L Y N S P S N P E N L	1249.5	-
188	L Y N S P S N F E N L	1299.5	-
189	L Y N S P S N Y E N L	1315.5	-
190	L Y N S P S N W E N L	1338.6	-
192	L Y N S P S Q P E N L	1263.5	-
193	L Y N S P S Q F E N L	1313.5	-
194	L Y N S P S Q Y E N L	1329.5	-
195	L Y N S P S Q W E N L	1352.6	-
197	L Y N S P S H P E N L	1272.5	-
198	L Y N S P S H F E N L	1322.5	-
199	L Y N S P S H Y E N L	1338.5	-
200	L Y N S P S H W E N L	1361.6	-
202	L Y G S P S M P E N L	1209.5	-
203	L Y G S P S M F E N L	1259.5	-
204	L Y G S P S M Y E N L	1275.5	-
205	L Y G S P S M W E N L	1298.6	-
207	L Y G S P S F P E N L	1225.4	-
208	L Y G S P S F F E N L	1275.4	-
209	L Y G S P S F Y E N L	1291.4	-
210	L Y G S P S F W E N L	1314.5	-
212	L Y G S P S Y P E N L	1241.4	-
213	L Y G S P S Y F E N L	1291.4	-
214	L Y G S P S Y Y E N L	1307.4	-
215	L Y G S P S Y W E N L	1330.5	-
217	L Y G S P S D P E N L	1193.3	-

218	L Y G S P S D F E N L	1243.3	-
219	L Y G S P S D Y E N L	1259.3	-
220	L Y G S P S D W E N L	1282.4	-
222	L Y G S P S E P E N L	1207.3	-
223	L Y G S P S E F E N L	1257.3	-
224	L Y G S P S E Y E N L	1273.3	-
225	L Y G S P S E W E N L	1296.4	-
227	L Y G S P S N P E N L	1192.4	-
228	L Y G S P S N F E N L	1242.4	-
229	L Y G S P S N Y E N L	1258.4	-
230	L Y G S P S N W E N L	1281.5	-
232	L Y G S P S Q P E N L	1206.4	-
233	L Y G S P S Q F E N L	1256.4	-
234	L Y G S P S Q Y E N L	1272.4	-
235	L Y G S P S Q W E N L	1295.5	-
237	L Y G S P S H P E N L	1215.4	-
238	L Y G S P S H F E N L	1265.4	-
239	L Y G S P S H Y E N L	1281.4	-
240	L Y G S P S H W E N L	1304.5	-
242	L Y A S P S M P E N L	1223.5	-
243	L Y A S P S M F E N L	1273.5	-
244	L Y A S P S M Y E N L	1289.5	-
245	L Y A S P S M W E N L	1312.6	-
247	L Y A S P S F P E N L	1239.4	-
248	L Y A S P S F F E N L	1289.4	-
249	L Y A S P S F Y E N L	1305.4	-
250	L Y A S P S F W E N L	1328.5	-
252	L Y A S P S Y P E N L	1255.4	-
253	L Y A S P S Y F E N L	1305.4	-
254	L Y A S P S Y Y E N L	1321.4	-
255	L Y A S P S Y W E N L	1344.5	-
257	L Y A S P S D P E N L	1207.3	-
258	L Y A S P S D F E N L	1257.3	-
259	L Y A S P S D Y E N L	1273.3	-
260	L Y A S P S D W E N L	1296.4	-
262	L Y A S P S E P E N L	1221.3	-

263	L Y A S P S E F E N L	1271.3	-
264	L Y A S P S E Y E N L	1287.3	-
265	L Y A S P S E W E N L	1310.4	-
267	L Y A S P S N P E N L	1206.4	-
268	L Y A S P S N F E N L	1256.4	-
269	L Y A S P S N Y E N L	1272.4	-
270	L Y A S P S N W E N L	1295.5	-
272	L Y A S P S Q P E N L	1220.4	-
273	L Y A S P S Q F E N L	1270.4	-
274	L Y A S P S Q Y E N L	1286.4	-
275	L Y A S P S Q W E N L	1309.5	-
277	L Y A S P S H P E N L	1229.4	-
278	L Y A S P S H F E N L	1279.4	-
279	L Y A S P S H Y E N L	1295.4	-
280	L Y A S P S H W E N L	1318.5	-
282	L F R S P S M P E N L	1292.6	-
283	L F R S P S M F E N L	1342.6	-
284	L F R S P S M Y E N L	1358.6	-
285	L F R S P S M W E N L	1381.7	-
287	L F R S P S F P E N L	1308.5	-
288	L F R S P S F F E N L	1358.5	-
289	L F R S P S F Y E N L	1374.5	-
290	L F R S P S F W E N L	1397.6	-
292	L F R S P S Y P E N L	1324.5	-
293	L F R S P S Y F E N L	1374.5	-
294	L F R S P S Y Y E N L	1390.5	-
295	L F R S P S Y W E N L	1413.6	-
297	L F R S P S D P E N L	1276.4	-
298	L F R S P S D F E N L	1326.4	-
299	L F R S P S D Y E N L	1342.4	-
300	L F R S P S D W E N L	1365.5	-
302	L F R S P S E P E N L	1290.4	-
303	L F R S P S E F E N L	1340.4	-
304	L F R S P S E Y E N L	1356.4	-
305	L F R S P S E W E N L	1379.5	-
307	L F R S P S N P E N L	1275.5	-

308	L F R S P S N F E N L	1325.5	-
309	L F R S P S N Y E N L	1341.5	-
310	L F R S P S N W E N L	1364.6	-
312	L F R S P S Q P E N L	1289.5	-
313	L F R S P S Q F E N L	1339.5	-
314	L F R S P S Q Y E N L	1355.5	-
315	L F R S P S Q W E N L	1378.6	-
317	L F R S P S H P E N L	1298.5	-
318	L F R S P S H F E N L	1348.5	-
319	L F R S P S H Y E N L	1364.5	-
320	L F R S P S H W E N L	1387.6	-
322	L F S S P S M P E N L	1224.3	-
323	L F S S P S M F E N L	1274.3	-
324	L F S S P S M Y E N L	1290.3	-
325	L F S S P S M W E N L	1313.4	-
327	L F S S P S F P E N L	1240.2	-
328	L F S S P S F F E N L	1290.2	-
329	L F S S P S F Y E N L	1306.2	-
330	L F S S P S F W E N L	1329.3	-
332	L F S S P S Y P E N L	1256.2	-
333	L F S S P S Y F E N L	1306.2	-
334	L F S S P S Y Y E N L	1322.2	-
335	L F S S P S Y W E N L	1345.3	-
337	L F S S P S D P E N L	1208.1	-
338	L F S S P S D F E N L	1258.1	-
339	L F S S P S D Y E N L	1274.1	-
340	L F S S P S D W E N L	1297.2	-
342	L F S S P S E P E N L	1222.1	-
343	L F S S P S E F E N L	1272.1	-
344	L F S S P S E Y E N L	1288.1	-
345	L F S S P S E W E N L	1311.2	-
347	L F S S P S N P E N L	1207.2	-
348	L F S S P S N F E N L	1257.2	-
349	L F S S P S N Y E N L	1273.2	-
350	L F S S P S N W E N L	1296.3	-
352	L F S S P S Q P E N L	1221.2	-

353	L F S S P S Q F E N L	1271.2	-
354	L F S S P S Q Y E N L	1287.2	-
355	L F S S P S Q W E N L	1310.3	-
357	L F S S P S H P E N L	1230.2	-
358	L F S S P S H F E N L	1280.2	-
359	L F S S P S H Y E N L	1296.2	-
360	L F S S P S H W E N L	1319.3	-
362	L F T S P S M P E N L	1237.5	-
363	L F T S P S M F E N L	1287.5	-
364	L F T S P S M Y E N L	1303.5	-
365	L F T S P S M W E N L	1326.6	-
367	L F T S P S F P E N L	1253.4	-
368	L F T S P S F F E N L	1303.4	-
369	L F T S P S F Y E N L	1319.4	-
370	L F T S P S F W E N L	1342.5	-
372	L F T S P S Y P E N L	1269.4	+
373	L F T S P S Y F E N L	1319.4	+
374	L F T S P S Y Y E N L	1335.4	+++
375	L F T S P S Y W E N L	1358.5	+
377	L F T S P S D P E N L	1221.3	-
378	L F T S P S D F E N L	1271.3	-
379	L F T S P S D Y E N L	1287.3	-
380	L F T S P S D W E N L	1310.4	-
382	L F T S P S E P E N L	1235.3	-
383	L F T S P S E F E N L	1285.3	-
384	L F T S P S E Y E N L	1301.3	-
385	L F T S P S E W E N L	1324.4	-
387	L F T S P S N P E N L	1220.4	-
388	L F T S P S N F E N L	1270.4	-
389	L F T S P S N Y E N L	1286.4	-
390	L F T S P S N W E N L	1309.5	-
392	L F T S P S Q P E N L	1234.4	-
393	L F T S P S Q F E N L	1284.4	-
394	L F T S P S Q Y E N L	1300.4	-
395	L F T S P S Q W E N L	1323.5	-
397	L F T S P S H P E N L	1243.4	-

398	L F T S P S H F E N L	1293.4	-
399	L F T S P S H Y E N L	1309.4	-
400	L F T S P S H W E N L	1332.5	-
402	L F H S P S M P E N L	1273.6	-
403	L F H S P S M F E N L	1323.6	-
404	L F H S P S M Y E N L	1339.6	-
405	L F H S P S M W E N L	1362.7	-
407	L F H S P S F P E N L	1289.5	-
408	L F H S P S F F E N L	1339.5	-
409	L F H S P S F Y E N L	1355.5	-
410	L F H S P S F W E N L	1378.6	-
412	L F H S P S Y P E N L	1305.5	-
413	L F H S P S Y F E N L	1355.5	-
414	L F H S P S Y Y E N L	1371.5	-
415	L F H S P S Y W E N L	1394.6	-
417	L F H S P S D P E N L	1257.4	-
418	L F H S P S D F E N L	1307.4	-
419	L F H S P S D Y E N L	1323.4	-
420	L F H S P S D W E N L	1346.5	-
422	L F H S P S E P E N L	1271.4	-
423	L F H S P S E F E N L	1321.4	-
424	L F H S P S E Y E N L	1337.4	-
425	L F H S P S E W E N L	1360.5	-
427	L F H S P S N P E N L	1256.5	-
428	L F H S P S N F E N L	1306.5	-
429	L F H S P S N Y E N L	1322.5	-
430	L F H S P S N W E N L	1345.6	-
432	L F H S P S Q P E N L	1270.5	-
433	L F H S P S Q F E N L	1320.5	-
434	L F H S P S Q Y E N L	1336.5	-
435	L F H S P S Q W E N L	1359.6	-
437	L F H S P S H P E N L	1279.5	-
438	L F H S P S H F E N L	1329.5	-
439	L F H S P S H Y E N L	1345.5	-
440	L F H S P S H W E N L	1368.6	-
442	L F N S P S M P E N L	1250.6	-

443 L F N S P S M F E N L	1300.6	-
444 L F N S P S M Y E N L	1316.6	-
445 L F N S P S M W E N L	1339.7	-
447 L F N S P S F P E N L	1266.5	-
448 L F N S P S F F E N L	1316.5	-
449 L F N S P S F Y E N L	1332.5	-
450 L F N S P S F W E N L	1355.6	-
452 L F N S P S Y P E N L	1282.5	-
453 L F N S P S Y F E N L	1332.5	-
454 L F N S P S Y Y E N L	1348.5	-
455 L F N S P S Y W E N L	1371.6	-
457 L F N S P S D P E N L	1234.4	-
458 L F N S P S D F E N L	1284.4	-
459 L F N S P S D Y E N L	1300.4	-
460 L F N S P S D W E N L	1323.5	-
462 L F N S P S E P E N L	1248.4	-
463 L F N S P S E F E N L	1298.4	-
464 L F N S P S E Y E N L	1314.4	-
465 L F N S P S E W E N L	1337.5	-
467 L F N S P S N P E N L	1233.5	-
468 L F N S P S N F E N L	1283.5	-
469 L F N S P S N Y E N L	1299.5	-
470 L F N S P S N W E N L	1322.6	-
472 L F N S P S Q P E N L	1247.5	-
473 L F N S P S Q F E N L	1297.5	-
474 L F N S P S Q Y E N L	1313.5	-
475 L F N S P S Q W E N L	1336.6	-
477 L F N S P S H P E N L	1256.5	-
478 L F N S P S H F E N L	1306.5	-
479 L F N S P S H Y E N L	1322.5	-
480 L F N S P S H W E N L	1345.6	-
482 L F G S P S M P E N L	1193.5	-
483 L F G S P S M F E N L	1243.5	-
484 L F G S P S M Y E N L	1259.5	-
485 L F G S P S M W E N L	1282.6	-
487 L F G S P S F P E N L	1209.4	-

488	L F G S P S F F E N L	1259.4	-
489	L F G S P S F Y E N L	1275.4	-
490	L F G S P S F W E N L	1298.5	-
492	L F G S P S Y P E N L	1225.4	-
493	L F G S P S Y F E N L	1275.4	-
494	L F G S P S Y Y E N L	1291.4	-
495	L F G S P S Y W E N L	1314.5	-
497	L F G S P S D P E N L	1177.3	-
498	L F G S P S D F E N L	1227.3	-
499	L F G S P S D Y E N L	1243.3	-
500	L F G S P S D W E N L	1266.4	-
502	L F G S P S E P E N L	1191.3	-
503	L F G S P S E F E N L	1241.3	-
504	L F G S P S E Y E N L	1257.3	-
505	L F G S P S E W E N L	1280.4	-
507	L F G S P S N P E N L	1176.4	-
508	L F G S P S N F E N L	1226.4	-
509	L F G S P S N Y E N L	1242.4	-
510	L F G S P S N W E N L	1265.5	-
512	L F G S P S Q P E N L	1190.4	-
513	L F G S P S Q F E N L	1240.4	-
514	L F G S P S Q Y E N L	1256.4	-
515	L F G S P S Q W E N L	1279.5	-
517	L F G S P S H P E N L	1199.4	-
518	L F G S P S H F E N L	1249.4	-
519	L F G S P S H Y E N L	1265.4	-
520	L F G S P S H W E N L	1288.5	-
522	L F A S P S M P E N L	1207.5	-
523	L F A S P S M F E N L	1257.5	-
524	L F A S P S M Y E N L	1273.5	-
525	L F A S P S M W E N L	1296.6	-
527	L F A S P S F P E N L	1223.4	-
528	L F A S P S F F E N L	1273.4	-
529	L F A S P S F Y E N L	1289.4	-
530	L F A S P S F W E N L	1312.5	-
532	L F A S P S Y P E N L	1239.4	-

533	L F A S P S Y F E N L	1289.4	-
534	L F A S P S Y Y E N L	1305.4	-
535	L F A S P S Y W E N L	1328.5	-
537	L F A S P S D P E N L	1191.3	-
538	L F A S P S D F E N L	1241.3	-
539	L F A S P S D Y E N L	1257.3	-
540	L F A S P S D W E N L	1280.4	-
542	L F A S P S E P E N L	1205.3	-
543	L F A S P S E F E N L	1255.3	-
544	L F A S P S E Y E N L	1271.3	-
545	L F A S P S E W E N L	1294.4	-
547	L F A S P S N P E N L	1190.4	-
548	L F A S P S N F E N L	1240.4	-
549	L F A S P S N Y E N L	1256.4	-
550	L F A S P S N W E N L	1279.5	-
552	L F A S P S Q P E N L	1204.4	-
553	L F A S P S Q F E N L	1254.4	-
554	L F A S P S Q Y E N L	1270.4	-
555	L F A S P S Q W E N L	1293.5	-
557	L F A S P S H P E N L	1213.4	-
558	L F A S P S H F E N L	1263.4	-
559	L F A S P S H Y E N L	1279.4	-
560	L F A S P S H W E N L	1302.5	-
562	F Y R S P S M P E N L	1342.6	-
563	F Y R S P S M F E N L	1392.6	-
564	F Y R S P S M Y E N L	1408.6	-
565	F Y R S P S M W E N L	1431.7	-
567	F Y R S P S F P E N L	1358.5	-
568	F Y R S P S F F E N L	1408.5	-
569	F Y R S P S F Y E N L	1424.5	-
570	F Y R S P S F W E N L	1447.6	-
572	F Y R S P S Y P E N L	1374.5	-
573	F Y R S P S Y F E N L	1424.5	-
574	F Y R S P S Y Y E N L	1440.5	-
575	F Y R S P S Y W E N L	1463.6	-
577	F Y R S P S D P E N L	1326.4	-

578	F Y R S P S D F E N L	1376.4	-
579	F Y R S P S D Y E N L	1392.4	-
580	F Y R S P S D W E N L	1415.5	-
582	F Y R S P S E P E N L	1340.4	-
583	F Y R S P S E F E N L	1390.4	-
584	F Y R S P S E Y E N L	1406.4	-
585	F Y R S P S E W E N L	1429.5	-
587	F Y R S P S N P E N L	1325.5	-
588	F Y R S P S N F E N L	1375.5	-
589	F Y R S P S N Y E N L	1391.5	-
590	F Y R S P S N W E N L	1414.6	-
592	F Y R S P S Q P E N L	1339.5	-
593	F Y R S P S Q F E N L	1389.5	-
594	F Y R S P S Q Y E N L	1405.5	-
595	F Y R S P S Q W E N L	1428.6	-
597	F Y R S P S H P E N L	1348.5	-
598	F Y R S P S H F E N L	1398.5	-
599	F Y R S P S H Y E N L	1414.5	-
600	F Y R S P S H W E N L	1437.6	-
602	F Y S S P S M P E N L	1274.3	-
603	F Y S S P S M F E N L	1324.3	-
604	F Y S S P S M Y E N L	1340.3	-
605	F Y S S P S M W E N L	1363.4	-
607	F Y S S P S F P E N L	1290.2	-
608	F Y S S P S F F E N L	1340.2	-
609	F Y S S P S F Y E N L	1356.2	-
610	F Y S S P S F W E N L	1379.3	-
612	F Y S S P S Y P E N L	1306.2	-
613	F Y S S P S Y F E N L	1356.2	-
614	F Y S S P S Y Y E N L	1372.2	-
615	F Y S S P S Y W E N L	1395.3	-
617	F Y S S P S D P E N L	1258.1	-
618	F Y S S P S D F E N L	1308.1	-
619	F Y S S P S D Y E N L	1324.1	-
620	F Y S S P S D W E N L	1347.2	-
622	F Y S S P S E P E N L	1272.1	-

623	F Y S S P S E F E N L	1322.1	-
624	F Y S S P S E Y E N L	1338.1	-
625	F Y S S P S E W E N L	1361.2	-
627	F Y S S P S N P E N L	1257.2	-
628	F Y S S P S N F E N L	1307.2	-
629	F Y S S P S N Y E N L	1323.2	-
630	F Y S S P S N W E N L	1346.3	-
632	F Y S S P S Q P E N L	1271.2	-
633	F Y S S P S Q F E N L	1321.2	-
634	F Y S S P S Q Y E N L	1337.2	-
635	F Y S S P S Q W E N L	1360.3	-
637	F Y S S P S H P E N L	1280.2	+
638	F Y S S P S H F E N L	1330.2	+
639	F Y S S P S H Y E N L	1346.2	+
640	F Y S S P S H W E N L	1369.3	+
642	F Y T S P S M P E N L	1287.5	+
643	F Y T S P S M F E N L	1337.5	+
644	F Y T S P S M Y E N L	1353.5	+
645	F Y T S P S M W E N L	1376.6	+
647	F Y T S P S F P E N L	1303.4	+
648	F Y T S P S F F E N L	1353.4	+
649	F Y T S P S F Y E N L	1369.4	+
650	F Y T S P S F W E N L	1392.5	+
652	F Y T S P S Y P E N L	1319.4	+
653	F Y T S P S Y F E N L	1369.4	+
654	F Y T S P S Y Y E N L	1385.4	+
655	F Y T S P S Y W E N L	1408.5	+
657	F Y T S P S D P E N L	1271.3	-
658	F Y T S P S D F E N L	1321.3	-
659	F Y T S P S D Y E N L	1337.3	-
660	F Y T S P S D W E N L	1360.4	-
662	F Y T S P S E P E N L	1285.3	-
663	F Y T S P S E F E N L	1335.3	-
664	F Y T S P S E Y E N L	1351.3	-
665	F Y T S P S E W E N L	1374.4	-
667	F Y T S P S N P E N L	1270.4	-

668	F Y T S P S N F E N L	1320.4	-
669	F Y T S P S N Y E N L	1336.4	-
670	F Y T S P S N W E N L	1359.5	-
672	F Y T S P S Q P E N L	1284.4	-
673	F Y T S P S Q F E N L	1334.4	-
674	F Y T S P S Q Y E N L	1350.4	-
675	F Y T S P S Q W E N L	1373.5	-
677	F Y T S P S H P E N L	1293.4	-
678	F Y T S P S H F E N L	1343.4	-
679	F Y T S P S H Y E N L	1359.4	-
680	F Y T S P S H W E N L	1382.5	-
682	F Y H S P S M P E N L	1323.6	-
683	F Y H S P S M F E N L	1373.6	-
684	F Y H S P S M Y E N L	1389.6	-
685	F Y H S P S M W E N L	1412.7	-
687	F Y H S P S F P E N L	1339.5	-
688	F Y H S P S F F E N L	1389.5	-
689	F Y H S P S F Y E N L	1405.5	-
690	F Y H S P S F W E N L	1428.6	-
692	F Y H S P S Y P E N L	1355.5	-
693	F Y H S P S Y F E N L	1405.5	-
694	F Y H S P S Y Y E N L	1421.5	-
695	F Y H S P S Y W E N L	1444.6	-
697	F Y H S P S D P E N L	1307.4	-
698	F Y H S P S D F E N L	1357.4	-
699	F Y H S P S D Y E N L	1373.4	-
700	F Y H S P S D W E N L	1396.5	-
702	F Y H S P S E P E N L	1321.4	-
703	F Y H S P S E F E N L	1371.4	-
704	F Y H S P S E Y E N L	1387.4	-
705	F Y H S P S E W E N L	1410.5	-
707	F Y H S P S N P E N L	1306.5	-
708	F Y H S P S N F E N L	1356.5	-
709	F Y H S P S N Y E N L	1372.5	-
710	F Y H S P S N W E N L	1395.6	-
712	F Y H S P S Q P E N L	1320.5	-

713	F Y H S P S Q F E N L	1370.5	-
714	F Y H S P S Q Y E N L	1386.5	-
715	F Y H S P S Q W E N L	1409.6	-
717	F Y H S P S H P E N L	1329.5	-
718	F Y H S P S H F E N L	1379.5	-
719	F Y H S P S H Y E N L	1395.5	-
720	F Y H S P S H W E N L	1418.6	-
722	F Y N S P S M P E N L	1300.6	-
723	F Y N S P S M F E N L	1350.6	-
724	F Y N S P S M Y E N L	1366.6	-
725	F Y N S P S M W E N L	1389.7	-
727	F Y N S P S F P E N L	1316.5	-
728	F Y N S P S F F E N L	1366.5	-
729	F Y N S P S F Y E N L	1382.5	-
730	F Y N S P S F W E N L	1405.6	-
732	F Y N S P S Y P E N L	1332.5	-
733	F Y N S P S Y F E N L	1382.5	-
734	F Y N S P S Y Y E N L	1398.5	-
735	F Y N S P S Y W E N L	1421.6	-
737	F Y N S P S D P E N L	1284.4	-
738	F Y N S P S D F E N L	1334.4	-
739	F Y N S P S D Y E N L	1350.4	-
740	F Y N S P S D W E N L	1373.5	-
742	F Y N S P S E P E N L	1298.4	-
743	F Y N S P S E F E N L	1348.4	-
744	F Y N S P S E Y E N L	1364.4	-
745	F Y N S P S E W E N L	1387.5	-
747	F Y N S P S N P E N L	1283.5	-
748	F Y N S P S N F E N L	1333.5	-
749	F Y N S P S N Y E N L	1349.5	-
750	F Y N S P S N W E N L	1372.6	-
752	F Y N S P S Q P E N L	1297.5	-
753	F Y N S P S Q F E N L	1347.5	-
754	F Y N S P S Q Y E N L	1363.5	-
755	F Y N S P S Q W E N L	1386.6	-
757	F Y N S P S H P E N L	1306.5	-

758	F Y N S P S H F E N L	1356.5	-
759	F Y N S P S H Y E N L	1372.5	-
760	F Y N S P S H W E N L	1395.6	-
762	F Y G S P S M P E N L	1243.5	-
763	F Y G S P S M F E N L	1293.5	-
764	F Y G S P S M Y E N L	1309.5	-
765	F Y G S P S M W E N L	1332.6	-
767	F Y G S P S F P E N L	1259.4	-
768	F Y G S P S F F E N L	1309.4	-
769	F Y G S P S F Y E N L	1325.4	-
770	F Y G S P S F W E N L	1348.5	-
772	F Y G S P S Y P E N L	1275.4	-
773	F Y G S P S Y F E N L	1325.4	-
774	F Y G S P S Y Y E N L	1341.4	-
775	F Y G S P S Y W E N L	1364.5	-
777	F Y G S P S D P E N L	1227.3	-
778	F Y G S P S D F E N L	1277.3	-
779	F Y G S P S D Y E N L	1293.3	-
780	F Y G S P S D W E N L	1316.4	-
782	F Y G S P S E P E N L	1241.3	-
783	F Y G S P S E F E N L	1291.3	-
784	F Y G S P S E Y E N L	1307.3	-
785	F Y G S P S E W E N L	1330.4	-
787	F Y G S P S N P E N L	1226.4	-
788	F Y G S P S N F E N L	1276.4	-
789	F Y G S P S N Y E N L	1292.4	-
790	F Y G S P S N W E N L	1315.5	-
792	F Y G S P S Q P E N L	1240.4	-
793	F Y G S P S Q F E N L	1290.4	-
794	F Y G S P S Q Y E N L	1306.4	-
795	F Y G S P S Q W E N L	1329.5	-
797	F Y G S P S H P E N L	1249.4	-
798	F Y G S P S H F E N L	1299.4	-
799	F Y G S P S H Y E N L	1315.4	-
800	F Y G S P S H W E N L	1338.5	-
802	F Y A S P S M P E N L	1257.5	-

803	F Y A S P S M F E N L	1307.5	-
804	F Y A S P S M Y E N L	1323.5	-
805	F Y A S P S M W E N L	1346.6	-
807	F Y A S P S F P E N L	1273.4	-
808	F Y A S P S F F E N L	1323.4	-
809	F Y A S P S F Y E N L	1339.4	-
810	F Y A S P S F W E N L	1362.5	-
812	F Y A S P S Y P E N L	1289.4	-
813	F Y A S P S Y F E N L	1339.4	-
814	F Y A S P S Y Y E N L	1355.4	-
815	F Y A S P S Y W E N L	1378.5	-
817	F Y A S P S D P E N L	1241.3	-
818	F Y A S P S D F E N L	1291.3	-
819	F Y A S P S D Y E N L	1307.3	-
820	F Y A S P S D W E N L	1330.4	-
822	F Y A S P S E P E N L	1255.3	-
823	F Y A S P S E F E N L	1305.3	-
824	F Y A S P S E Y E N L	1321.3	-
825	F Y A S P S E W E N L	1344.4	-
827	F Y A S P S N P E N L	1240.4	-
828	F Y A S P S N F E N L	1290.4	-
829	F Y A S P S N Y E N L	1306.4	-
830	F Y A S P S N W E N L	1329.5	-
832	F Y A S P S Q P E N L	1254.4	-
833	F Y A S P S Q F E N L	1304.4	-
834	F Y A S P S Q Y E N L	1320.4	-
835	F Y A S P S Q W E N L	1343.5	-
837	F Y A S P S H P E N L	1263.4	-
838	F Y A S P S H F E N L	1313.4	-
839	F Y A S P S H Y E N L	1329.4	-
840	F Y A S P S H W E N L	1352.5	-
842	F F R S P S M P E N L	1326.6	-
843	F F R S P S M F E N L	1376.6	-
844	F F R S P S M Y E N L	1392.6	-
845	F F R S P S M W E N L	1415.7	-
847	F F R S P S F P E N L	1342.5	-

848	F F R S P S F F E N L	1392.5	-
849	F F R S P S F Y E N L	1408.5	-
850	F F R S P S F W E N L	1431.6	-
852	F F R S P S Y P E N L	1358.5	-
853	F F R S P S Y F E N L	1408.5	-
854	F F R S P S Y Y E N L	1424.5	-
855	F F R S P S Y W E N L	1447.6	-
857	F F R S P S D P E N L	1310.4	-
858	F F R S P S D F E N L	1360.4	-
859	F F R S P S D Y E N L	1376.4	-
860	F F R S P S D W E N L	1399.5	-
862	F F R S P S E P E N L	1324.4	-
863	F F R S P S E F E N L	1374.4	-
864	F F R S P S E Y E N L	1390.4	-
865	F F R S P S E W E N L	1413.5	-
867	F F R S P S N P E N L	1309.5	-
868	F F R S P S N F E N L	1359.5	-
869	F F R S P S N Y E N L	1375.5	-
870	F F R S P S N W E N L	1398.6	-
872	F F R S P S Q P E N L	1323.5	-
873	F F R S P S Q F E N L	1373.5	-
874	F F R S P S Q Y E N L	1389.5	-
875	F F R S P S Q W E N L	1412.6	-
877	F F R S P S H P E N L	1332.5	-
878	F F R S P S H F E N L	1382.5	-
879	F F R S P S H Y E N L	1398.5	-
880	F F R S P S H W E N L	1421.6	-
882	F F S S P S M P E N L	1258.3	-
883	F F S S P S M F E N L	1308.3	-
884	F F S S P S M Y E N L	1324.3	-
885	F F S S P S M W E N L	1347.4	-
887	F F S S P S F P E N L	1274.2	-
888	F F S S P S F F E N L	1324.2	-
889	F F S S P S F Y E N L	1340.2	-
890	F F S S P S F W E N L	1363.3	-
892	F F S S P S Y P E N L	1290.2	-

893	F F S S P S Y F E N L	1340.2	-
894	F F S S P S Y Y E N L	1356.2	-
895	F F S S P S Y W E N L	1379.3	-
897	F F S S P S D P E N L	1242.1	-
898	F F S S P S D F E N L	1292.1	-
899	F F S S P S D Y E N L	1308.1	-
900	F F S S P S D W E N L	1331.2	-
902	F F S S P S E P E N L	1256.1	-
903	F F S S P S E F E N L	1306.1	-
904	F F S S P S E Y E N L	1322.1	-
905	F F S S P S E W E N L	1345.2	-
907	F F S S P S N P E N L	1241.2	-
908	F F S S P S N F E N L	1291.2	-
909	F F S S P S N Y E N L	1307.2	-
910	F F S S P S N W E N L	1330.3	-
912	F F S S P S Q P E N L	1255.2	-
913	F F S S P S Q F E N L	1305.2	-
914	F F S S P S Q Y E N L	1321.2	-
915	F F S S P S Q W E N L	1344.3	-
917	F F S S P S H P E N L	1264.2	-
918	F F S S P S H F E N L	1314.2	-
919	F F S S P S H Y E N L	1330.2	-
920	F F S S P S H W E N L	1353.3	-
922	F F T S P S M P E N L	1271.5	-
923	F F T S P S M F E N L	1321.5	-
924	F F T S P S M Y E N L	1337.5	-
925	F F T S P S M W E N L	1360.6	-
927	F F T S P S F P E N L	1287.4	-
928	F F T S P S F F E N L	1337.4	-
929	F F T S P S F Y E N L	1353.4	-
930	F F T S P S F W E N L	1376.5	-
932	F F T S P S Y P E N L	1303.4	-
933	F F T S P S Y F E N L	1353.4	-
934	F F T S P S Y Y E N L	1369.4	-
935	F F T S P S Y W E N L	1392.5	-
937	F F T S P S D P E N L	1255.3	-

938	F F T S P S D F E N L	1305.3	-
939	F F T S P S D Y E N L	1321.3	-
940	F F T S P S D W E N L	1344.4	-
942	F F T S P S E P E N L	1269.3	-
943	F F T S P S E F E N L	1319.3	-
944	F F T S P S E Y E N L	1335.3	-
945	F F T S P S E W E N L	1358.4	-
947	F F T S P S N P E N L	1254.4	-
948	F F T S P S N F E N L	1304.4	-
949	F F T S P S N Y E N L	1320.4	-
950	F F T S P S N W E N L	1343.5	-
952	F F T S P S Q P E N L	1268.4	-
953	F F T S P S Q F E N L	1318.4	-
954	F F T S P S Q Y E N L	1334.4	-
955	F F T S P S Q W E N L	1357.5	-
957	F F T S P S H P E N L	1277.4	-
958	F F T S P S H F E N L	1327.4	-
959	F F T S P S H Y E N L	1343.4	-
960	F F T S P S H W E N L	1366.5	-
962	F F H S P S M P E N L	1307.6	-
963	F F H S P S M F E N L	1357.6	-
964	F F H S P S M Y E N L	1373.6	-
965	F F H S P S M W E N L	1396.7	-
967	F F H S P S F P E N L	1323.5	-
968	F F H S P S F F E N L	1373.5	-
969	F F H S P S F Y E N L	1389.5	-
970	F F H S P S F W E N L	1412.6	-
972	F F H S P S Y P E N L	1339.5	-
973	F F H S P S Y F E N L	1389.5	-
974	F F H S P S Y Y E N L	1405.5	-
975	F F H S P S Y W E N L	1428.6	-
977	F F H S P S D P E N L	1291.4	-
978	F F H S P S D F E N L	1341.4	-
979	F F H S P S D Y E N L	1357.4	-
980	F F H S P S D W E N L	1380.5	-
982	F F H S P S E P E N L	1305.4	-

983	F F H S P S E F E N L	1355.4	-
984	F F H S P S E Y E N L	1371.4	-
985	F F H S P S E W E N L	1394.5	-
987	F F H S P S N P E N L	1290.5	-
988	F F H S P S N F E N L	1340.5	-
989	F F H S P S N Y E N L	1356.5	-
990	F F H S P S N W E N L	1379.6	-
992	F F H S P S Q P E N L	1304.5	-
993	F F H S P S Q F E N L	1354.5	-
994	F F H S P S Q Y E N L	1370.5	-
995	F F H S P S Q W E N L	1393.6	-
997	F F H S P S H P E N L	1313.5	-
998	F F H S P S H F E N L	1363.5	-
999	F F H S P S H Y E N L	1379.5	-
1000	F F H S P S H W E N L	1402.6	-
1002	F F N S P S M P E N L	1284.6	-
1003	F F N S P S M F E N L	1334.6	-
1004	F F N S P S M Y E N L	1350.6	-
1005	F F N S P S M W E N L	1373.7	-
1007	F F N S P S F P E N L	1300.5	-
1008	F F N S P S F F E N L	1350.5	-
1009	F F N S P S F Y E N L	1366.5	-
1010	F F N S P S F W E N L	1389.6	-
1012	F F N S P S Y P E N L	1316.5	-
1013	F F N S P S Y F E N L	1366.5	-
1014	F F N S P S Y Y E N L	1382.5	-
1015	F F N S P S Y W E N L	1405.6	-
1017	F F N S P S D P E N L	1268.4	-
1018	F F N S P S D F E N L	1318.4	-
1019	F F N S P S D Y E N L	1334.4	-
1020	F F N S P S D W E N L	1357.5	-
1022	F F N S P S E P E N L	1282.4	-
1023	F F N S P S E F E N L	1332.4	-
1024	F F N S P S E Y E N L	1348.4	-
1025	F F N S P S E W E N L	1371.5	-
1027	F F N S P S N P E N L	1267.5	-

1028	F F N S P S N F E N L	1317.5	-
1029	F F N S P S N Y E N L	1333.5	-
1030	F F N S P S N W E N L	1356.6	-
1032	F F N S P S Q P E N L	1281.5	-
1033	F F N S P S Q F E N L	1331.5	-
1034	F F N S P S Q Y E N L	1347.5	-
1035	F F N S P S Q W E N L	1370.6	-
1037	F F N S P S H P E N L	1290.5	-
1038	F F N S P S H F E N L	1340.5	-
1039	F F N S P S H Y E N L	1356.5	-
1040	F F N S P S H W E N L	1379.6	-
1042	F F G S P S M P E N L	1227.5	-
1043	F F G S P S M F E N L	1277.5	-
1044	F F G S P S M Y E N L	1293.5	-
1045	F F G S P S M W E N L	1316.6	-
1047	F F G S P S F P E N L	1243.4	-
1048	F F G S P S F F E N L	1293.4	-
1049	F F G S P S F Y E N L	1309.4	-
1050	F F G S P S F W E N L	1332.5	-
1052	F F G S P S Y P E N L	1259.4	-
1053	F F G S P S Y F E N L	1309.4	-
1054	F F G S P S Y Y E N L	1325.4	-
1055	F F G S P S Y W E N L	1348.5	-
1057	F F G S P S D P E N L	1211.3	-
1058	F F G S P S D F E N L	1261.3	-
1059	F F G S P S D Y E N L	1277.3	-
1060	F F G S P S D W E N L	1300.4	-
1062	F F G S P S E P E N L	1225.3	-
1063	F F G S P S E F E N L	1275.3	-
1064	F F G S P S E Y E N L	1291.3	-
1065	F F G S P S E W E N L	1314.4	-
1067	F F G S P S N P E N L	1210.4	-
1068	F F G S P S N F E N L	1260.4	-
1069	F F G S P S N Y E N L	1276.4	-
1070	F F G S P S N W E N L	1299.5	-
1072	F F G S P S Q P E N L	1224.4	-

1073	F F G S P S Q F E N L	1274.4	-
1074	F F G S P S Q Y E N L	1290.4	-
1075	F F G S P S Q W E N L	1313.5	-
1077	F F G S P S H P E N L	1233.4	-
1078	F F G S P S H F E N L	1283.4	-
1079	F F G S P S H Y E N L	1299.4	-
1080	F F G S P S H W E N L	1322.5	-
1082	F F A S P S M P E N L	1241.5	-
1083	F F A S P S M F E N L	1291.5	-
1084	F F A S P S M Y E N L	1307.5	-
1085	F F A S P S M W E N L	1330.6	-
1087	F F A S P S F P E N L	1257.4	-
1088	F F A S P S F F E N L	1307.4	-
1089	F F A S P S F Y E N L	1323.4	-
1090	F F A S P S F W E N L	1346.5	-
1092	F F A S P S Y P E N L	1273.4	-
1093	F F A S P S Y F E N L	1323.4	-
1094	F F A S P S Y Y E N L	1339.4	-
1095	F F A S P S Y W E N L	1362.5	-
1097	F F A S P S D P E N L	1225.3	-
1098	F F A S P S D F E N L	1275.3	-
1099	F F A S P S D Y E N L	1291.3	-
1100	F F A S P S D W E N L	1314.4	-
1102	F F A S P S E P E N L	1239.3	-
1103	F F A S P S E F E N L	1289.3	-
1104	F F A S P S E Y E N L	1305.3	-
1105	F F A S P S E W E N L	1328.4	-
1107	F F A S P S N P E N L	1224.4	-
1108	F F A S P S N F E N L	1274.4	-
1109	F F A S P S N Y E N L	1290.4	-
1110	F F A S P S N W E N L	1313.5	-
1112	F F A S P S Q P E N L	1238.4	-
1113	F F A S P S Q F E N L	1288.4	-
1114	F F A S P S Q Y E N L	1304.4	-
1115	F F A S P S Q W E N L	1327.5	-
1117	F F A S P S H P E N L	1247.4	-

1118	F F A S P S H F E N L	1297.4	-
1119	F F A S P S H Y E N L	1313.4	-
1120	F F A S P S H W E N L	1336.5	-
1122	W Y R S P S M P E N L	1381.7	+
1123	W Y R S P S M F E N L	1431.7	+
1124	W Y R S P S M Y E N L	1447.7	++
1125	W Y R S P S M W E N L	1470.8	++
1127	W Y R S P S F P E N L	1397.6	++
1128	W Y R S P S F F E N L	1447.6	++
1129	W Y R S P S F Y E N L	1463.6	+++
1130	W Y R S P S F W E N L	1486.7	++
1132	W Y R S P S Y P E N L	1413.6	++
1133	W Y R S P S Y F E N L	1463.6	+
1134	W Y R S P S Y Y E N L	1479.6	++
1135	W Y R S P S Y W E N L	1502.7	+
1137	W Y R S P S D P E N L	1365.5	-
1138	W Y R S P S D F E N L	1415.5	-
1139	W Y R S P S D Y E N L	1431.5	-
1140	W Y R S P S D W E N L	1454.6	-
1142	W Y R S P S E P E N L	1379.5	-
1143	W Y R S P S E F E N L	1429.5	-
1144	W Y R S P S E Y E N L	1445.5	-
1145	W Y R S P S E W E N L	1468.6	-
1147	W Y R S P S N P E N L	1364.6	-
1148	W Y R S P S N F E N L	1414.6	-
1149	W Y R S P S N Y E N L	1430.6	-
1150	W Y R S P S N W E N L	1453.7	-
1152	W Y R S P S Q P E N L	1378.6	-
1153	W Y R S P S Q F E N L	1428.6	-
1154	W Y R S P S Q Y E N L	1444.6	-
1155	W Y R S P S Q W E N L	1467.7	-
1157	W Y R S P S H P E N L	1387.6	-
1158	W Y R S P S H F E N L	1437.6	-
1159	W Y R S P S H Y E N L	1453.6	-
1160	W Y R S P S H W E N L	1476.7	-
1162	W Y S S P S M P E N L	1313.4	-

1163	W Y S S P S M F E N L	1363.4	-
1164	W Y S S P S M Y E N L	1379.4	-
1165	W Y S S P S M W E N L	1402.5	-
1167	W Y S S P S F P E N L	1329.3	-
1168	W Y S S P S F F E N L	1379.3	-
1169	W Y S S P S F Y E N L	1395.3	-
1170	W Y S S P S F W E N L	1418.4	-
1172	W Y S S P S Y P E N L	1345.3	-
1173	W Y S S P S Y F E N L	1395.3	-
1174	W Y S S P S Y Y E N L	1411.3	-
1175	W Y S S P S Y W E N L	1434.4	-
1177	W Y S S P S D P E N L	1297.2	-
1178	W Y S S P S D F E N L	1347.2	-
1179	W Y S S P S D Y E N L	1363.2	-
1180	W Y S S P S D W E N L	1386.3	-
1182	W Y S S P S E P E N L	1311.2	-
1183	W Y S S P S E F E N L	1361.2	-
1184	W Y S S P S E Y E N L	1377.2	-
1185	W Y S S P S E W E N L	1400.3	-
1187	W Y S S P S N P E N L	1296.3	-
1188	W Y S S P S N F E N L	1346.3	-
1189	W Y S S P S N Y E N L	1362.3	-
1190	W Y S S P S N W E N L	1385.4	-
1192	W Y S S P S Q P E N L	1310.3	-
1193	W Y S S P S Q F E N L	1360.3	-
1194	W Y S S P S Q Y E N L	1376.3	-
1195	W Y S S P S Q W E N L	1399.4	-
1197	W Y S S P S H P E N L	1319.3	-
1198	W Y S S P S H F E N L	1369.3	-
1199	W Y S S P S H Y E N L	1385.3	-
1200	W Y S S P S H W E N L	1408.4	-
1202	W Y T S P S M P E N L	1326.6	+
1203	W Y T S P S M F E N L	1376.6	+
1204	W Y T S P S M Y E N L	1392.6	+
1205	W Y T S P S M W E N L	1415.7	+
1207	W Y T S P S F P E N L	1342.5	+

1208	W Y T S P S F F E N L	1392.5	+
1209	W Y T S P S F Y E N L	1408.5	+
1210	W Y T S P S F W E N L	1431.6	+
1212	W Y T S P S Y P E N L	1358.5	++
1213	W Y T S P S Y F E N L	1408.5	+
1214	W Y T S P S Y Y E N L	1424.5	+
1215	W Y T S P S Y W E N L	1447.6	+
1217	W Y T S P S D P E N L	1310.4	-
1218	W Y T S P S D F E N L	1360.4	-
1219	W Y T S P S D Y E N L	1376.4	-
1220	W Y T S P S D W E N L	1399.5	-
1222	W Y T S P S E P E N L	1324.4	-
1223	W Y T S P S E F E N L	1374.4	-
1224	W Y T S P S E Y E N L	1390.4	-
1225	W Y T S P S E W E N L	1413.5	-
1227	W Y T S P S N P E N L	1309.5	-
1228	W Y T S P S N F E N L	1359.5	-
1229	W Y T S P S N Y E N L	1375.5	-
1230	W Y T S P S N W E N L	1398.6	-
1232	W Y T S P S Q P E N L	1323.5	-
1233	W Y T S P S Q F E N L	1373.5	-
1234	W Y T S P S Q Y E N L	1389.5	-
1235	W Y T S P S Q W E N L	1412.6	-
1237	W Y T S P S H P E N L	1332.5	+
1238	W Y T S P S H F E N L	1382.5	+
1239	W Y T S P S H Y E N L	1398.5	+
1240	W Y T S P S H W E N L	1421.6	+
1242	W Y H S P S M P E N L	1362.7	-
1243	W Y H S P S M F E N L	1412.7	-
1244	W Y H S P S M Y E N L	1428.7	-
1245	W Y H S P S M W E N L	1451.8	-
1247	W Y H S P S F P E N L	1378.6	-
1248	W Y H S P S F F E N L	1428.6	-
1249	W Y H S P S F Y E N L	1444.6	-
1250	W Y H S P S F W E N L	1467.7	-
1252	W Y H S P S Y P E N L	1394.6	-

1253	W Y H S P S Y F E N L	1444.6	-
1254	W Y H S P S Y Y E N L	1460.6	-
1255	W Y H S P S Y W E N L	1483.7	-
1257	W Y H S P S D P E N L	1346.5	-
1258	W Y H S P S D F E N L	1396.5	-
1259	W Y H S P S D Y E N L	1412.5	-
1260	W Y H S P S D W E N L	1435.6	-
1262	W Y H S P S E P E N L	1360.5	-
1263	W Y H S P S E F E N L	1410.5	-
1264	W Y H S P S E Y E N L	1426.5	-
1265	W Y H S P S E W E N L	1449.6	-
1267	W Y H S P S N P E N L	1345.6	-
1268	W Y H S P S N F E N L	1395.6	-
1269	W Y H S P S N Y E N L	1411.6	-
1270	W Y H S P S N W E N L	1434.7	-
1272	W Y H S P S Q P E N L	1359.6	-
1273	W Y H S P S Q F E N L	1409.6	-
1274	W Y H S P S Q Y E N L	1425.6	-
1275	W Y H S P S Q W E N L	1448.7	-
1277	W Y H S P S H P E N L	1368.6	-
1278	W Y H S P S H F E N L	1418.6	-
1279	W Y H S P S H Y E N L	1434.6	-
1280	W Y H S P S H W E N L	1457.7	-
1282	W Y N S P S M P E N L	1339.7	-
1283	W Y N S P S M F E N L	1389.7	-
1284	W Y N S P S M Y E N L	1405.7	-
1285	W Y N S P S M W E N L	1428.8	-
1287	W Y N S P S F P E N L	1355.6	-
1288	W Y N S P S F F E N L	1405.6	-
1289	W Y N S P S F Y E N L	1421.6	-
1290	W Y N S P S F W E N L	1444.7	-
1292	W Y N S P S Y P E N L	1371.6	-
1293	W Y N S P S Y F E N L	1421.6	-
1294	W Y N S P S Y Y E N L	1437.6	-
1295	W Y N S P S Y W E N L	1460.7	-
1297	W Y N S P S D P E N L	1323.5	-

1298	W Y N S P S D F E N L	1373.5	-
1299	W Y N S P S D Y E N L	1389.5	-
1300	W Y N S P S D W E N L	1412.6	-
1302	W Y N S P S E P E N L	1337.5	-
1303	W Y N S P S E F E N L	1387.5	-
1304	W Y N S P S E Y E N L	1403.5	-
1305	W Y N S P S E W E N L	1426.6	-
1307	W Y N S P S N P E N L	1322.6	-
1308	W Y N S P S N F E N L	1372.6	-
1309	W Y N S P S N Y E N L	1388.6	-
1310	W Y N S P S N W E N L	1411.7	-
1312	W Y N S P S Q P E N L	1336.6	-
1313	W Y N S P S Q F E N L	1386.6	-
1314	W Y N S P S Q Y E N L	1402.6	-
1315	W Y N S P S Q W E N L	1425.7	-
1317	W Y N S P S H P E N L	1345.6	-
1318	W Y N S P S H F E N L	1395.6	-
1319	W Y N S P S H Y E N L	1411.6	-
1320	W Y N S P S H W E N L	1434.7	-
1322	W Y G S P S M P E N L	1282.6	-
1323	W Y G S P S M F E N L	1332.6	-
1324	W Y G S P S M Y E N L	1348.6	-
1325	W Y G S P S M W E N L	1371.7	-
1327	W Y G S P S F P E N L	1298.5	-
1328	W Y G S P S F F E N L	1348.5	-
1329	W Y G S P S F Y E N L	1364.5	-
1330	W Y G S P S F W E N L	1387.6	-
1332	W Y G S P S Y P E N L	1314.5	-
1333	W Y G S P S Y F E N L	1364.5	-
1334	W Y G S P S Y Y E N L	1380.5	-
1335	W Y G S P S Y W E N L	1403.6	-
1337	W Y G S P S D P E N L	1266.4	-
1338	W Y G S P S D F E N L	1316.4	-
1339	W Y G S P S D Y E N L	1332.4	-
1340	W Y G S P S D W E N L	1355.5	-
1342	W Y G S P S E P E N L	1280.4	-

1343	W Y G S P S E F E N L	1330.4	-
1344	W Y G S P S E Y E N L	1346.4	-
1345	W Y G S P S E W E N L	1369.5	-
1347	W Y G S P S N P E N L	1265.5	-
1348	W Y G S P S N F E N L	1315.5	-
1349	W Y G S P S N Y E N L	1331.5	-
1350	W Y G S P S N W E N L	1354.6	-
1352	W Y G S P S Q P E N L	1279.5	-
1353	W Y G S P S Q F E N L	1329.5	-
1354	W Y G S P S Q Y E N L	1345.5	-
1355	W Y G S P S Q W E N L	1368.6	-
1357	W Y G S P S H P E N L	1288.5	-
1358	W Y G S P S H F E N L	1338.5	-
1359	W Y G S P S H Y E N L	1354.5	-
1360	W Y G S P S H W E N L	1377.6	-
1362	W Y A S P S M P E N L	1296.6	-
1363	W Y A S P S M F E N L	1346.6	-
1364	W Y A S P S M Y E N L	1362.6	-
1365	W Y A S P S M W E N L	1385.7	-
1367	W Y A S P S F P E N L	1312.5	-
1368	W Y A S P S F F E N L	1362.5	-
1369	W Y A S P S F Y E N L	1378.5	-
1370	W Y A S P S F W E N L	1401.6	-
1372	W Y A S P S Y P E N L	1328.5	-
1373	W Y A S P S Y F E N L	1378.5	-
1374	W Y A S P S Y Y E N L	1394.5	-
1375	W Y A S P S Y W E N L	1417.6	-
1377	W Y A S P S D P E N L	1280.4	-
1378	W Y A S P S D F E N L	1330.4	-
1379	W Y A S P S D Y E N L	1346.4	-
1380	W Y A S P S D W E N L	1369.5	-
1382	W Y A S P S E P E N L	1294.4	-
1383	W Y A S P S E F E N L	1344.4	-
1384	W Y A S P S E Y E N L	1360.4	-
1385	W Y A S P S E W E N L	1383.5	-
1387	W Y A S P S N P E N L	1279.5	-

1388	W Y A S P S N F E N L	1329.5	-
1389	W Y A S P S N Y E N L	1345.5	-
1390	W Y A S P S N W E N L	1368.6	-
1392	W Y A S P S Q P E N L	1293.5	-
1393	W Y A S P S Q F E N L	1343.5	-
1394	W Y A S P S Q Y E N L	1359.5	-
1395	W Y A S P S Q W E N L	1382.6	-
1397	W Y A S P S H P E N L	1302.5	-
1398	W Y A S P S H F E N L	1352.5	-
1399	W Y A S P S H Y E N L	1368.5	-
1400	W Y A S P S H W E N L	1391.6	-
1402	W F R S P S M P E N L	1365.7	-
1403	W F R S P S M F E N L	1415.7	-
1404	W F R S P S M Y E N L	1431.7	-
1405	W F R S P S M W E N L	1454.8	-
1407	W F R S P S F P E N L	1381.6	-
1408	W F R S P S F F E N L	1431.6	-
1409	W F R S P S F Y E N L	1447.6	-
1410	W F R S P S F W E N L	1470.7	-
1412	W F R S P S Y P E N L	1397.6	-
1413	W F R S P S Y F E N L	1447.6	-
1414	W F R S P S Y Y E N L	1463.6	-
1415	W F R S P S Y W E N L	1486.7	-
1417	W F R S P S D P E N L	1349.5	-
1418	W F R S P S D F E N L	1399.5	-
1419	W F R S P S D Y E N L	1415.5	-
1420	W F R S P S D W E N L	1438.6	-
1422	W F R S P S E P E N L	1363.5	-
1423	W F R S P S E F E N L	1413.5	-
1424	W F R S P S E Y E N L	1429.5	-
1425	W F R S P S E W E N L	1452.6	-
1427	W F R S P S N P E N L	1348.6	-
1428	W F R S P S N F E N L	1398.6	-
1429	W F R S P S N Y E N L	1414.6	-
1430	W F R S P S N W E N L	1437.7	-
1432	W F R S P S Q P E N L	1362.6	-

1433	W F R S P S Q F E N L	1412.6	-
1434	W F R S P S Q Y E N L	1428.6	-
1435	W F R S P S Q W E N L	1451.7	-
1437	W F R S P S H P E N L	1371.6	-
1438	W F R S P S H F E N L	1421.6	-
1439	W F R S P S H Y E N L	1437.6	-
1440	W F R S P S H W E N L	1460.7	-
1442	W F S S P S M P E N L	1297.4	-
1443	W F S S P S M F E N L	1347.4	-
1444	W F S S P S M Y E N L	1363.4	-
1445	W F S S P S M W E N L	1386.5	-
1447	W F S S P S F P E N L	1313.3	-
1448	W F S S P S F F E N L	1363.3	-
1449	W F S S P S F Y E N L	1379.3	-
1450	W F S S P S F W E N L	1402.4	-
1452	W F S S P S Y P E N L	1329.3	-
1453	W F S S P S Y F E N L	1379.3	-
1454	W F S S P S Y Y E N L	1395.3	-
1455	W F S S P S Y W E N L	1418.4	-
1457	W F S S P S D P E N L	1281.2	-
1458	W F S S P S D F E N L	1331.2	-
1459	W F S S P S D Y E N L	1347.2	-
1460	W F S S P S D W E N L	1370.3	-
1462	W F S S P S E P E N L	1295.2	-
1463	W F S S P S E F E N L	1345.2	-
1464	W F S S P S E Y E N L	1361.2	-
1465	W F S S P S E W E N L	1384.3	-
1467	W F S S P S N P E N L	1280.3	-
1468	W F S S P S N F E N L	1330.3	-
1469	W F S S P S N Y E N L	1346.3	-
1470	W F S S P S N W E N L	1369.4	-
1472	W F S S P S Q P E N L	1294.3	-
1473	W F S S P S Q F E N L	1344.3	-
1474	W F S S P S Q Y E N L	1360.3	-
1475	W F S S P S Q W E N L	1383.4	-
1477	W F S S P S H P E N L	1303.3	-

1478	W F S S P S H F E N L	1353.3	-
1479	W F S S P S H Y E N L	1369.3	-
1480	W F S S P S H W E N L	1392.4	-
1482	W F T S P S M P E N L	1310.6	-
1483	W F T S P S M F E N L	1360.6	-
1484	W F T S P S M Y E N L	1376.6	-
1485	W F T S P S M W E N L	1399.7	-
1487	W F T S P S F P E N L	1326.5	-
1488	W F T S P S F F E N L	1376.5	-
1489	W F T S P S F Y E N L	1392.5	-
1490	W F T S P S F W E N L	1415.6	-
1492	W F T S P S Y P E N L	1342.5	-
1493	W F T S P S Y F E N L	1392.5	-
1494	W F T S P S Y Y E N L	1408.5	-
1495	W F T S P S Y W E N L	1431.6	-
1497	W F T S P S D P E N L	1294.4	-
1498	W F T S P S D F E N L	1344.4	-
1499	W F T S P S D Y E N L	1360.4	-
1500	W F T S P S D W E N L	1383.5	-
1502	W F T S P S E P E N L	1308.4	-
1503	W F T S P S E F E N L	1358.4	-
1504	W F T S P S E Y E N L	1374.4	-
1505	W F T S P S E W E N L	1397.5	-
1507	W F T S P S N P E N L	1293.5	-
1508	W F T S P S N F E N L	1343.5	-
1509	W F T S P S N Y E N L	1359.5	-
1510	W F T S P S N W E N L	1382.6	-
1512	W F T S P S Q P E N L	1307.5	-
1513	W F T S P S Q F E N L	1357.5	-
1514	W F T S P S Q Y E N L	1373.5	-
1515	W F T S P S Q W E N L	1396.6	-
1517	W F T S P S H P E N L	1316.5	-
1518	W F T S P S H F E N L	1366.5	-
1519	W F T S P S H Y E N L	1382.5	-
1520	W F T S P S H W E N L	1405.6	-
1522	W F H S P S M P E N L	1346.7	-

1523	W F H S P S M F E N L	1396.7	-
1524	W F H S P S M Y E N L	1412.7	-
1525	W F H S P S M W E N L	1435.8	-
1527	W F H S P S F P E N L	1362.6	-
1528	W F H S P S F F E N L	1412.6	-
1529	W F H S P S F Y E N L	1428.6	-
1530	W F H S P S F W E N L	1451.7	-
1532	W F H S P S Y P E N L	1378.6	-
1533	W F H S P S Y F E N L	1428.6	-
1534	W F H S P S Y Y E N L	1444.6	-
1535	W F H S P S Y W E N L	1467.7	-
1537	W F H S P S D P E N L	1330.5	-
1538	W F H S P S D F E N L	1380.5	-
1539	W F H S P S D Y E N L	1396.5	-
1540	W F H S P S D W E N L	1419.6	-
1542	W F H S P S E P E N L	1344.5	-
1543	W F H S P S E F E N L	1394.5	-
1544	W F H S P S E Y E N L	1410.5	-
1545	W F H S P S E W E N L	1433.6	-
1547	W F H S P S N P E N L	1329.6	-
1548	W F H S P S N F E N L	1379.6	-
1549	W F H S P S N Y E N L	1395.6	-
1550	W F H S P S N W E N L	1418.7	-
1552	W F H S P S Q P E N L	1343.6	-
1553	W F H S P S Q F E N L	1393.6	-
1554	W F H S P S Q Y E N L	1409.6	-
1555	W F H S P S Q W E N L	1432.7	-
1557	W F H S P S H P E N L	1352.6	-
1558	W F H S P S H F E N L	1402.6	-
1559	W F H S P S H Y E N L	1418.6	-
1560	W F H S P S H W E N L	1441.7	-
1562	W F N S P S M P E N L	1323.7	-
1563	W F N S P S M F E N L	1373.7	-
1564	W F N S P S M Y E N L	1389.7	-
1565	W F N S P S M W E N L	1412.8	-
1567	W F N S P S F P E N L	1339.6	-

1568	WFNSPSFFENL	1389.6	-
1569	WFNSPSFYENL	1405.6	-
1570	WFNSPSFWENL	1428.7	-
1572	WFNSPSYPENL	1355.6	-
1573	WFNSPSYFENL	1405.6	-
1574	WFNSPSYYENL	1421.6	-
1575	WFNSPSYWENL	1444.7	-
1577	WFNSPSDPENL	1307.5	-
1578	WFNSPSDFENL	1357.5	-
1579	WFNSPSDYENL	1373.5	-
1580	WFNSPSDWENL	1396.6	-
1582	WFNSPSEPENL	1321.5	-
1583	WFNSPSEFENL	1371.5	-
1584	WFNSPSEYENL	1387.5	-
1585	WFNSPSEWENL	1410.6	-
1587	WFNSPSNPENL	1306.6	-
1588	WFNSPSNFENL	1356.6	-
1589	WFNSPSNYENL	1372.6	-
1590	WFNSPSNWENL	1395.7	-
1592	WFNSPSQPENL	1320.6	-
1593	WFNSPSQFENL	1370.6	-
1594	WFNSPSQYENL	1386.6	-
1595	WFNSPSQWENL	1409.7	-
1597	WFNSPSHPENL	1329.6	-
1598	WFNSPSHFENL	1379.6	-
1599	WFNSPSHYENL	1395.6	-
1600	WFNSPSHWENL	1418.7	-
1602	WFGSPSMPENL	1266.6	-
1603	WFGSPSMFENL	1316.6	-
1604	WFGSPSMYENL	1332.6	-
1605	WFGSPSMWENL	1355.7	-
1607	WFGSPSFPENL	1282.5	-
1608	WFGSPSFFENL	1332.5	-
1609	WFGSPSFYENL	1348.5	-
1610	WFGSPSFWENL	1371.6	-
1612	WFGSPSYPENL	1298.5	-

1613	W F G S P S Y F E N L	1348.5	-
1614	W F G S P S Y Y E N L	1364.5	-
1615	W F G S P S Y W E N L	1387.6	-
1617	W F G S P S D P E N L	1250.4	-
1618	W F G S P S D F E N L	1300.4	-
1619	W F G S P S D Y E N L	1316.4	-
1620	W F G S P S D W E N L	1339.5	-
1622	W F G S P S E P E N L	1264.4	-
1623	W F G S P S E F E N L	1314.4	-
1624	W F G S P S E Y E N L	1330.4	-
1625	W F G S P S E W E N L	1353.5	-
1627	W F G S P S N P E N L	1249.5	-
1628	W F G S P S N F E N L	1299.5	-
1629	W F G S P S N Y E N L	1315.5	-
1630	W F G S P S N W E N L	1338.6	-
1632	W F G S P S Q P E N L	1263.5	-
1633	W F G S P S Q F E N L	1313.5	-
1634	W F G S P S Q Y E N L	1329.5	-
1635	W F G S P S Q W E N L	1352.6	-
1637	W F G S P S H P E N L	1272.5	-
1638	W F G S P S H F E N L	1322.5	-
1639	W F G S P S H Y E N L	1338.5	-
1640	W F G S P S H W E N L	1361.6	-
1642	W F A S P S M P E N L	1280.6	-
1643	W F A S P S M F E N L	1330.6	-
1644	W F A S P S M Y E N L	1346.6	-
1645	W F A S P S M W E N L	1369.7	-
1647	W F A S P S F P E N L	1296.5	-
1648	W F A S P S F F E N L	1346.5	-
1649	W F A S P S F Y E N L	1362.5	-
1650	W F A S P S F W E N L	1385.6	-
1652	W F A S P S Y P E N L	1312.5	-
1653	W F A S P S Y F E N L	1362.5	-
1654	W F A S P S Y Y E N L	1378.5	-
1655	W F A S P S Y W E N L	1401.6	-
1657	W F A S P S D P E N L	1264.4	-

1658	W F A S P S D F E N L	1314.4	-
1659	W F A S P S D Y E N L	1330.4	-
1660	W F A S P S D W E N L	1353.5	-
1662	W F A S P S E P E N L	1278.4	-
1663	W F A S P S E F E N L	1328.4	-
1664	W F A S P S E Y E N L	1344.4	-
1665	W F A S P S E W E N L	1367.5	-
1667	W F A S P S N P E N L	1263.5	-
1668	W F A S P S N F E N L	1313.5	-
1669	W F A S P S N Y E N L	1329.5	-
1670	W F A S P S N W E N L	1352.6	-
1672	W F A S P S Q P E N L	1277.5	-
1673	W F A S P S Q F E N L	1327.5	-
1674	W F A S P S Q Y E N L	1343.5	-
1675	W F A S P S Q W E N L	1366.6	-
1677	W F A S P S H P E N L	1286.5	-
1678	W F A S P S H F E N L	1336.5	-
1679	W F A S P S H Y E N L	1352.5	-
1680	W F A S P S H W E N L	1375.6	-
1682	M Y R S P S M P E N L	1326.7	-
1683	M Y R S P S M F E N L	1376.7	-
1684	M Y R S P S M Y E N L	1392.7	-
1685	M Y R S P S M W E N L	1415.8	-
1687	M Y R S P S F P E N L	1342.6	-
1688	M Y R S P S F F E N L	1392.6	-
1689	M Y R S P S F Y E N L	1408.6	-
1690	M Y R S P S F W E N L	1431.7	-
1692	M Y R S P S Y P E N L	1358.6	-
1693	M Y R S P S Y F E N L	1408.6	-
1694	M Y R S P S Y Y E N L	1424.6	-
1695	M Y R S P S Y W E N L	1447.7	-
1697	M Y R S P S D P E N L	1310.5	-
1698	M Y R S P S D F E N L	1360.5	-
1699	M Y R S P S D Y E N L	1376.5	-
1700	M Y R S P S D W E N L	1399.6	-
1702	M Y R S P S E P E N L	1324.5	-

1703	M Y R S P S E F E N L	1374.5	-
1704	M Y R S P S E Y E N L	1390.5	-
1705	M Y R S P S E W E N L	1413.6	-
1707	M Y R S P S N P E N L	1309.6	-
1708	M Y R S P S N F E N L	1359.6	-
1709	M Y R S P S N Y E N L	1375.6	-
1710	M Y R S P S N W E N L	1398.7	-
1712	M Y R S P S Q P E N L	1323.6	-
1713	M Y R S P S Q F E N L	1373.6	-
1714	M Y R S P S Q Y E N L	1389.6	-
1715	M Y R S P S Q W E N L	1412.7	-
1717	M Y R S P S H P E N L	1332.6	-
1718	M Y R S P S H F E N L	1382.6	-
1719	M Y R S P S H Y E N L	1398.6	-
1720	M Y R S P S H W E N L	1421.7	-
1722	M Y S S P S M P E N L	1258.4	-
1723	M Y S S P S M F E N L	1308.4	-
1724	M Y S S P S M Y E N L	1324.4	-
1725	M Y S S P S M W E N L	1347.5	-
1727	M Y S S P S F P E N L	1274.3	-
1728	M Y S S P S F F E N L	1324.3	-
1729	M Y S S P S F Y E N L	1340.3	-
1730	M Y S S P S F W E N L	1363.4	-
1732	M Y S S P S Y P E N L	1290.3	-
1733	M Y S S P S Y F E N L	1340.3	-
1734	M Y S S P S Y Y E N L	1356.3	-
1735	M Y S S P S Y W E N L	1379.4	-
1737	M Y S S P S D P E N L	1242.2	-
1738	M Y S S P S D F E N L	1292.2	-
1739	M Y S S P S D Y E N L	1308.2	-
1740	M Y S S P S D W E N L	1331.3	-
1742	M Y S S P S E P E N L	1256.2	-
1743	M Y S S P S E F E N L	1306.2	-
1744	M Y S S P S E Y E N L	1322.2	-
1745	M Y S S P S E W E N L	1345.3	-
1747	M Y S S P S N P E N L	1241.3	-

1748	M Y S S P S N F E N L	1291.3	-
1749	M Y S S P S N Y E N L	1307.3	-
1750	M Y S S P S N W E N L	1330.4	-
1752	M Y S S P S Q P E N L	1255.3	-
1753	M Y S S P S Q F E N L	1305.3	-
1754	M Y S S P S Q Y E N L	1321.3	-
1755	M Y S S P S Q W E N L	1344.4	-
1757	M Y S S P S H P E N L	1264.3	-
1758	M Y S S P S H F E N L	1314.3	-
1759	M Y S S P S H Y E N L	1330.3	-
1760	M Y S S P S H W E N L	1353.4	-
1762	M Y T S P S M P E N L	1271.6	-
1763	M Y T S P S M F E N L	1321.6	-
1764	M Y T S P S M Y E N L	1337.6	-
1765	M Y T S P S M W E N L	1360.7	-
1767	M Y T S P S F P E N L	1287.5	-
1768	M Y T S P S F F E N L	1337.5	-
1769	M Y T S P S F Y E N L	1353.5	-
1770	M Y T S P S F W E N L	1376.6	-
1772	M Y T S P S Y P E N L	1303.5	-
1773	M Y T S P S Y F E N L	1353.5	-
1774	M Y T S P S Y Y E N L	1369.5	-
1775	M Y T S P S Y W E N L	1392.6	-
1777	M Y T S P S D P E N L	1255.4	-
1778	M Y T S P S D F E N L	1305.4	-
1779	M Y T S P S D Y E N L	1321.4	-
1780	M Y T S P S D W E N L	1344.5	-
1782	M Y T S P S E P E N L	1269.4	-
1783	M Y T S P S E F E N L	1319.4	-
1784	M Y T S P S E Y E N L	1335.4	-
1785	M Y T S P S E W E N L	1358.5	-
1787	M Y T S P S N P E N L	1254.5	-
1788	M Y T S P S N F E N L	1304.5	-
1789	M Y T S P S N Y E N L	1320.5	-
1790	M Y T S P S N W E N L	1343.6	-
1792	M Y T S P S Q P E N L	1268.5	-

1793	M Y T S P S Q F E N L	1318.5	-
1794	M Y T S P S Q Y E N L	1334.5	-
1795	M Y T S P S Q W E N L	1357.6	-
1797	M Y T S P S H P E N L	1277.5	-
1798	M Y T S P S H F E N L	1327.5	-
1799	M Y T S P S H Y E N L	1343.5	-
1800	M Y T S P S H W E N L	1366.6	-
1802	M Y H S P S M P E N L	1307.7	-
1803	M Y H S P S M F E N L	1357.7	-
1804	M Y H S P S M Y E N L	1373.7	-
1805	M Y H S P S M W E N L	1396.8	-
1807	M Y H S P S F P E N L	1323.6	-
1808	M Y H S P S F F E N L	1373.6	-
1809	M Y H S P S F Y E N L	1389.6	-
1810	M Y H S P S F W E N L	1412.7	-
1812	M Y H S P S Y P E N L	1339.6	-
1813	M Y H S P S Y F E N L	1389.6	-
1814	M Y H S P S Y Y E N L	1405.6	-
1815	M Y H S P S Y W E N L	1428.7	-
1817	M Y H S P S D P E N L	1291.5	-
1818	M Y H S P S D F E N L	1341.5	-
1819	M Y H S P S D Y E N L	1357.5	-
1820	M Y H S P S D W E N L	1380.6	-
1822	M Y H S P S E P E N L	1305.5	-
1823	M Y H S P S E F E N L	1355.5	-
1824	M Y H S P S E Y E N L	1371.5	-
1825	M Y H S P S E W E N L	1394.6	-
1827	M Y H S P S N P E N L	1290.6	-
1828	M Y H S P S N F E N L	1340.6	-
1829	M Y H S P S N Y E N L	1356.6	-
1830	M Y H S P S N W E N L	1379.7	-
1832	M Y H S P S Q P E N L	1304.6	-
1833	M Y H S P S Q F E N L	1354.6	-
1834	M Y H S P S Q Y E N L	1370.6	-
1835	M Y H S P S Q W E N L	1393.7	-
1837	M Y H S P S H P E N L	1313.6	-

1838	M Y H S P S H F E N L	1363.6	-
1839	M Y H S P S H Y E N L	1379.6	-
1840	M Y H S P S H W E N L	1402.7	-
1842	M Y N S P S M P E N L	1284.7	-
1843	M Y N S P S M F E N L	1334.7	-
1844	M Y N S P S M Y E N L	1350.7	-
1845	M Y N S P S M W E N L	1373.8	-
1847	M Y N S P S F P E N L	1300.6	-
1848	M Y N S P S F F E N L	1350.6	-
1849	M Y N S P S F Y E N L	1366.6	-
1850	M Y N S P S F W E N L	1389.7	-
1852	M Y N S P S Y P E N L	1316.6	-
1853	M Y N S P S Y F E N L	1366.6	-
1854	M Y N S P S Y Y E N L	1382.6	-
1855	M Y N S P S Y W E N L	1405.7	-
1857	M Y N S P S D P E N L	1268.5	-
1858	M Y N S P S D F E N L	1318.5	-
1859	M Y N S P S D Y E N L	1334.5	-
1860	M Y N S P S D W E N L	1357.6	-
1862	M Y N S P S E P E N L	1282.5	-
1863	M Y N S P S E F E N L	1332.5	-
1864	M Y N S P S E Y E N L	1348.5	-
1865	M Y N S P S E W E N L	1371.6	-
1867	M Y N S P S N P E N L	1267.6	-
1868	M Y N S P S N F E N L	1317.6	-
1869	M Y N S P S N Y E N L	1333.6	-
1870	M Y N S P S N W E N L	1356.7	-
1872	M Y N S P S Q P E N L	1281.6	-
1873	M Y N S P S Q F E N L	1331.6	-
1874	M Y N S P S Q Y E N L	1347.6	-
1875	M Y N S P S Q W E N L	1370.7	-
1877	M Y N S P S H P E N L	1290.6	-
1878	M Y N S P S H F E N L	1340.6	-
1879	M Y N S P S H Y E N L	1356.6	-
1880	M Y N S P S H W E N L	1379.7	-
1882	M Y G S P S M P E N L	1227.6	-

1883	M Y G S P S M F E N L	1277.6	-
1884	M Y G S P S M Y E N L	1293.6	-
1885	M Y G S P S M W E N L	1316.7	-
1887	M Y G S P S F P E N L	1243.5	-
1888	M Y G S P S F F E N L	1293.5	-
1889	M Y G S P S F Y E N L	1309.5	-
1890	M Y G S P S F W E N L	1332.6	-
1892	M Y G S P S Y P E N L	1259.5	-
1893	M Y G S P S Y F E N L	1309.5	-
1894	M Y G S P S Y Y E N L	1325.5	-
1895	M Y G S P S Y W E N L	1348.6	-
1897	M Y G S P S D P E N L	1211.4	-
1898	M Y G S P S D F E N L	1261.4	-
1899	M Y G S P S D Y E N L	1277.4	-
1900	M Y G S P S D W E N L	1300.5	-
1902	M Y G S P S E P E N L	1225.4	-
1903	M Y G S P S E F E N L	1275.4	-
1904	M Y G S P S E Y E N L	1291.4	-
1905	M Y G S P S E W E N L	1314.5	-
1907	M Y G S P S N P E N L	1210.5	-
1908	M Y G S P S N F E N L	1260.5	-
1909	M Y G S P S N Y E N L	1276.5	-
1910	M Y G S P S N W E N L	1299.6	-
1912	M Y G S P S Q P E N L	1224.5	-
1913	M Y G S P S Q F E N L	1274.5	-
1914	M Y G S P S Q Y E N L	1290.5	-
1915	M Y G S P S Q W E N L	1313.6	-
1917	M Y G S P S H P E N L	1233.5	-
1918	M Y G S P S H F E N L	1283.5	-
1919	M Y G S P S H Y E N L	1299.5	-
1920	M Y G S P S H W E N L	1322.6	-
1922	M Y A S P S M P E N L	1241.6	-
1923	M Y A S P S M F E N L	1291.6	-
1924	M Y A S P S M Y E N L	1307.6	-
1925	M Y A S P S M W E N L	1330.7	-
1927	M Y A S P S F P E N L	1257.5	-

1928	M Y A S P S F F E N L	1307.5	-
1929	M Y A S P S F Y E N L	1323.5	-
1930	M Y A S P S F W E N L	1346.6	-
1932	M Y A S P S Y P E N L	1273.5	-
1933	M Y A S P S Y F E N L	1323.5	-
1934	M Y A S P S Y Y E N L	1339.5	-
1935	M Y A S P S Y W E N L	1362.6	-
1937	M Y A S P S D P E N L	1225.4	-
1938	M Y A S P S D F E N L	1275.4	-
1939	M Y A S P S D Y E N L	1291.4	-
1940	M Y A S P S D W E N L	1314.5	-
1942	M Y A S P S E P E N L	1239.4	-
1943	M Y A S P S E F E N L	1289.4	-
1944	M Y A S P S E Y E N L	1305.4	-
1945	M Y A S P S E W E N L	1328.5	-
1947	M Y A S P S N P E N L	1224.5	-
1948	M Y A S P S N F E N L	1274.5	-
1949	M Y A S P S N Y E N L	1290.5	-
1950	M Y A S P S N W E N L	1313.6	-
1952	M Y A S P S Q P E N L	1238.5	-
1953	M Y A S P S Q F E N L	1288.5	-
1954	M Y A S P S Q Y E N L	1304.5	-
1955	M Y A S P S Q W E N L	1327.6	-
1957	M Y A S P S H P E N L	1247.5	-
1958	M Y A S P S H F E N L	1297.5	-
1959	M Y A S P S H Y E N L	1313.5	-
1960	M Y A S P S H W E N L	1336.6	-
1962	M F R S P S M P E N L	1310.7	-
1963	M F R S P S M F E N L	1360.7	-
1964	M F R S P S M Y E N L	1376.7	-
1965	M F R S P S M W E N L	1399.8	-
1967	M F R S P S F P E N L	1326.6	-
1968	M F R S P S F F E N L	1376.6	-
1969	M F R S P S F Y E N L	1392.6	-
1970	M F R S P S F W E N L	1415.7	-
1972	M F R S P S Y P E N L	1342.6	-

1973	M F R S P S Y F E N L	1392.6	-
1974	M F R S P S Y Y E N L	1408.6	-
1975	M F R S P S Y W E N L	1431.7	-
1977	M F R S P S D P E N L	1294.5	-
1978	M F R S P S D F E N L	1344.5	-
1979	M F R S P S D Y E N L	1360.5	-
1980	M F R S P S D W E N L	1383.6	-
1982	M F R S P S E P E N L	1308.5	-
1983	M F R S P S E F E N L	1358.5	-
1984	M F R S P S E Y E N L	1374.5	-
1985	M F R S P S E W E N L	1397.6	-
1987	M F R S P S N P E N L	1293.6	-
1988	M F R S P S N F E N L	1343.6	-
1989	M F R S P S N Y E N L	1359.6	-
1990	M F R S P S N W E N L	1382.7	-
1992	M F R S P S Q P E N L	1307.6	-
1993	M F R S P S Q F E N L	1357.6	-
1994	M F R S P S Q Y E N L	1373.6	-
1995	M F R S P S Q W E N L	1396.7	-
1997	M F R S P S H P E N L	1316.6	-
1998	M F R S P S H F E N L	1366.6	-
1999	M F R S P S H Y E N L	1382.6	-
2000	M F R S P S H W E N L	1405.7	-
2002	M F S S P S M P E N L	1242.4	-
2003	M F S S P S M F E N L	1292.4	-
2004	M F S S P S M Y E N L	1308.4	-
2005	M F S S P S M W E N L	1331.5	-
2007	M F S S P S F P E N L	1258.3	-
2008	M F S S P S F F E N L	1308.3	-
2009	M F S S P S F Y E N L	1324.3	-
2010	M F S S P S F W E N L	1347.4	-
2012	M F S S P S Y P E N L	1274.3	-
2013	M F S S P S Y F E N L	1324.3	-
2014	M F S S P S Y Y E N L	1340.3	-
2015	M F S S P S Y W E N L	1363.4	-
2017	M F S S P S D P E N L	1226.2	-

2018	M F S S P S D F E N L	1276.2	-
2019	M F S S P S D Y E N L	1292.2	-
2020	M F S S P S D W E N L	1315.3	-
2022	M F S S P S E P E N L	1240.2	-
2023	M F S S P S E F E N L	1290.2	-
2024	M F S S P S E Y E N L	1306.2	-
2025	M F S S P S E W E N L	1329.3	-
2027	M F S S P S N P E N L	1225.3	-
2028	M F S S P S N F E N L	1275.3	-
2029	M F S S P S N Y E N L	1291.3	-
2030	M F S S P S N W E N L	1314.4	-
2032	M F S S P S Q P E N L	1239.3	-
2033	M F S S P S Q F E N L	1289.3	-
2034	M F S S P S Q Y E N L	1305.3	-
2035	M F S S P S Q W E N L	1328.4	-
2037	M F S S P S H P E N L	1248.3	-
2038	M F S S P S H F E N L	1298.3	-
2039	M F S S P S H Y E N L	1314.3	-
2040	M F S S P S H W E N L	1337.4	-
2042	M F T S P S M P E N L	1255.6	-
2043	M F T S P S M F E N L	1305.6	-
2044	M F T S P S M Y E N L	1321.6	-
2045	M F T S P S M W E N L	1344.7	-
2047	M F T S P S F P E N L	1271.5	-
2048	M F T S P S F F E N L	1321.5	-
2049	M F T S P S F Y E N L	1337.5	-
2050	M F T S P S F W E N L	1360.6	-
2052	M F T S P S Y P E N L	1287.5	-
2053	M F T S P S Y F E N L	1337.5	-
2054	M F T S P S Y Y E N L	1353.5	-
2055	M F T S P S Y W E N L	1376.6	-
2057	M F T S P S D P E N L	1239.4	-
2058	M F T S P S D F E N L	1289.4	-
2059	M F T S P S D Y E N L	1305.4	-
2060	M F T S P S D W E N L	1328.5	-
2062	M F T S P S E P E N L	1253.4	-

2063	M F T S P S E F E N L	1303.4	-
2064	M F T S P S E Y E N L	1319.4	-
2065	M F T S P S E W E N L	1342.5	-
2067	M F T S P S N P E N L	1238.5	-
2068	M F T S P S N F E N L	1288.5	-
2069	M F T S P S N Y E N L	1304.5	-
2070	M F T S P S N W E N L	1327.6	-
2072	M F T S P S Q P E N L	1252.5	-
2073	M F T S P S Q F E N L	1302.5	-
2074	M F T S P S Q Y E N L	1318.5	-
2075	M F T S P S Q W E N L	1341.6	-
2077	M F T S P S H P E N L	1261.5	-
2078	M F T S P S H F E N L	1311.5	-
2079	M F T S P S H Y E N L	1327.5	-
2080	M F T S P S H W E N L	1350.6	-
2082	M F H S P S M P E N L	1291.7	-
2083	M F H S P S M F E N L	1341.7	-
2084	M F H S P S M Y E N L	1357.7	-
2085	M F H S P S M W E N L	1380.8	-
2087	M F H S P S F P E N L	1307.6	-
2088	M F H S P S F F E N L	1357.6	-
2089	M F H S P S F Y E N L	1373.6	-
2090	M F H S P S F W E N L	1396.7	-
2092	M F H S P S Y P E N L	1323.6	-
2093	M F H S P S Y F E N L	1373.6	-
2094	M F H S P S Y Y E N L	1389.6	-
2095	M F H S P S Y W E N L	1412.7	-
2097	M F H S P S D P E N L	1275.5	-
2098	M F H S P S D F E N L	1325.5	-
2099	M F H S P S D Y E N L	1341.5	-
2100	M F H S P S D W E N L	1364.6	-
2102	M F H S P S E P E N L	1289.5	-
2103	M F H S P S E F E N L	1339.5	-
2104	M F H S P S E Y E N L	1355.5	-
2105	M F H S P S E W E N L	1378.6	-
2107	M F H S P S N P E N L	1274.6	-

2108	M F H S P S N F E N L	1324.6	-
2109	M F H S P S N Y E N L	1340.6	-
2110	M F H S P S N W E N L	1363.7	-
2112	M F H S P S Q P E N L	1288.6	-
2113	M F H S P S Q F E N L	1338.6	-
2114	M F H S P S Q Y E N L	1354.6	-
2115	M F H S P S Q W E N L	1377.7	-
2117	M F H S P S H P E N L	1297.6	-
2118	M F H S P S H F E N L	1347.6	-
2119	M F H S P S H Y E N L	1363.6	-
2120	M F H S P S H W E N L	1386.7	-
2122	M F N S P S M P E N L	1268.7	-
2123	M F N S P S M F E N L	1318.7	-
2124	M F N S P S M Y E N L	1334.7	-
2125	M F N S P S M W E N L	1357.8	-
2127	M F N S P S F P E N L	1284.6	-
2128	M F N S P S F F E N L	1334.6	-
2129	M F N S P S F Y E N L	1350.6	-
2130	M F N S P S F W E N L	1373.7	-
2132	M F N S P S Y P E N L	1300.6	-
2133	M F N S P S Y F E N L	1350.6	-
2134	M F N S P S Y Y E N L	1366.6	-
2135	M F N S P S Y W E N L	1389.7	-
2137	M F N S P S D P E N L	1252.5	-
2138	M F N S P S D F E N L	1302.5	-
2139	M F N S P S D Y E N L	1318.5	-
2140	M F N S P S D W E N L	1341.6	-
2142	M F N S P S E P E N L	1266.5	-
2143	M F N S P S E F E N L	1316.5	-
2144	M F N S P S E Y E N L	1332.5	-
2145	M F N S P S E W E N L	1355.6	-
2147	M F N S P S N P E N L	1251.6	-
2148	M F N S P S N F E N L	1301.6	-
2149	M F N S P S N Y E N L	1317.6	-
2150	M F N S P S N W E N L	1340.7	-
2152	M F N S P S Q P E N L	1265.6	-

2153	M F N S P S Q F E N L	1315.6	-
2154	M F N S P S Q Y E N L	1331.6	-
2155	M F N S P S Q W E N L	1354.7	-
2157	M F N S P S H P E N L	1274.6	-
2158	M F N S P S H F E N L	1324.6	-
2159	M F N S P S H Y E N L	1340.6	-
2160	M F N S P S H W E N L	1363.7	-
2162	M F G S P S M P E N L	1211.6	-
2163	M F G S P S M F E N L	1261.6	-
2164	M F G S P S M Y E N L	1277.6	-
2165	M F G S P S M W E N L	1300.7	-
2167	M F G S P S F P E N L	1227.5	-
2168	M F G S P S F F E N L	1277.5	-
2169	M F G S P S F Y E N L	1293.5	-
2170	M F G S P S F W E N L	1316.6	-
2172	M F G S P S Y P E N L	1243.5	-
2173	M F G S P S Y F E N L	1293.5	-
2174	M F G S P S Y Y E N L	1309.5	-
2175	M F G S P S Y W E N L	1332.6	-
2177	M F G S P S D P E N L	1195.4	-
2178	M F G S P S D F E N L	1245.4	-
2179	M F G S P S D Y E N L	1261.4	-
2180	M F G S P S D W E N L	1284.5	-
2182	M F G S P S E P E N L	1209.4	-
2183	M F G S P S E F E N L	1259.4	-
2184	M F G S P S E Y E N L	1275.4	-
2185	M F G S P S E W E N L	1298.5	-
2187	M F G S P S N P E N L	1194.5	-
2188	M F G S P S N F E N L	1244.5	-
2189	M F G S P S N Y E N L	1260.5	-
2190	M F G S P S N W E N L	1283.6	-
2192	M F G S P S Q P E N L	1208.5	-
2193	M F G S P S Q F E N L	1258.5	-
2194	M F G S P S Q Y E N L	1274.5	-
2195	M F G S P S Q W E N L	1297.6	-
2197	M F G S P S H P E N L	1217.5	-

2198	M F G S P S H F E N L	1267.5	-
2199	M F G S P S H Y E N L	1283.5	-
2200	M F G S P S H W E N L	1306.6	-
2202	M F A S P S M P E N L	1225.6	-
2203	M F A S P S M F E N L	1275.6	-
2204	M F A S P S M Y E N L	1291.6	-
2205	M F A S P S M W E N L	1314.7	-
2207	M F A S P S F P E N L	1241.5	-
2208	M F A S P S F F E N L	1291.5	-
2209	M F A S P S F Y E N L	1307.5	-
2210	M F A S P S F W E N L	1330.6	-
2212	M F A S P S Y P E N L	1257.5	-
2213	M F A S P S Y F E N L	1307.5	-
2214	M F A S P S Y Y E N L	1323.5	-
2215	M F A S P S Y W E N L	1346.6	-
2217	M F A S P S D P E N L	1209.4	-
2218	M F A S P S D F E N L	1259.4	-
2219	M F A S P S D Y E N L	1275.4	-
2220	M F A S P S D W E N L	1298.5	-
2222	M F A S P S E P E N L	1223.4	-
2223	M F A S P S E F E N L	1273.4	-
2224	M F A S P S E Y E N L	1289.4	-
2225	M F A S P S E W E N L	1312.5	-
2227	M F A S P S N P E N L	1208.5	-
2228	M F A S P S N F E N L	1258.5	-
2229	M F A S P S N Y E N L	1274.5	-
2230	M F A S P S N W E N L	1297.6	-
2232	M F A S P S Q P E N L	1222.5	-
2233	M F A S P S Q F E N L	1272.5	-
2234	M F A S P S Q Y E N L	1288.5	-
2235	M F A S P S Q W E N L	1311.6	-
2237	M F A S P S H P E N L	1231.5	-
2238	M F A S P S H F E N L	1281.5	-
2239	M F A S P S H Y E N L	1297.5	-
2240	M F A S P S H W E N L	1320.6	-
2242	R Y S L P P E L S N M	1308.6	-

2243	A Y R S P S M P E N L	1266.5	-
2244	R Y R S P S M P E N L	1351.6	-
2245	N Y R S P S M P E N L	1309.6	-
2246	D Y R S P S M P E N L	1310.5	-
2247	C Y R S P S M P E N L	1298.6	-
2248	Q Y R S P S M P E N L	1323.6	-
2249	E Y R S P S M P E N L	1324.5	-
2250	G Y R S P S M P E N L	1252.5	-
2251	H Y R S P S M P E N L	1332.6	-
2252	I Y R S P S M P E N L	1308.6	-
2253	L Y R S P S M P E N L	1308.6	-
2254	K Y R S P S M P E N L	1323.6	-
2255	M Y R S P S M P E N L	1326.7	-
2256	F Y R S P S M P E N L	1342.6	-
2257	P Y R S P S M P E N L	1292.6	-
2258	S Y R S P S M P E N L	1283.3	-
2259	T Y R S P S M P E N L	1296.5	-
2260	W Y R S P S M P E N L	1381.7	-
2261	Y Y R S P S M P E N L	1358.6	-
2262	V Y R S P S M P E N L	1294.6	-
2263	L A R S P S M P E N L	1216.5	-
2264	L R R S P S M P E N L	1301.6	-
2265	L N R S P S M P E N L	1259.6	-
2266	L D R S P S M P E N L	1260.5	-
2267	L C R S P S M P E N L	1248.6	-
2268	L Q R S P S M P E N L	1273.6	-
2269	L E R S P S M P E N L	1274.5	-
2270	L G R S P S M P E N L	1202.5	-
2271	L H R S P S M P E N L	1282.6	-
2272	L I R S P S M P E N L	1258.6	-
2273	L L R S P S M P E N L	1258.6	-
2274	L K R S P S M P E N L	1273.6	+
2275	L M R S P S M P E N L	1276.7	-
2276	L F R S P S M P E N L	1292.6	-
2277	L P R S P S M P E N L	1242.6	-
2278	L S R S P S M P E N L	1233.3	-

2279	L T R S P S M P E N L	1246.5	-
2280	L W R S P S M P E N L	1331.7	-
2281	L Y R S P S M P E N L	1308.6	-
2282	L V R S P S M P E N L	1244.6	-
2283	L Y A S P S M P E N L	1223.5	-
2284	L Y R S P S M P E N L	1308.6	-
2285	L Y N S P S M P E N L	1266.6	-
2286	L Y D S P S M P E N L	1267.5	-
2287	L Y C S P S M P E N L	1255.6	-
2288	L Y Q S P S M P E N L	1280.6	-
2289	L Y E S P S M P E N L	1281.5	-
2290	L Y G S P S M P E N L	1209.5	-
2291	L Y H S P S M P E N L	1289.6	-
2292	L Y I S P S M P E N L	1265.6	+
2293	L Y L S P S M P E N L	1265.6	-
2294	L Y K S P S M P E N L	1280.6	-
2295	L Y M S P S M P E N L	1283.7	-
2296	L Y F S P S M P E N L	1299.6	-
2297	L Y P S P S M P E N L	1249.6	-
2298	L Y S S P S M P E N L	1240.3	-
2299	L Y T S P S M P E N L	1253.5	-
2300	L Y W S P S M P E N L	1338.7	-
2301	L Y Y S P S M P E N L	1315.6	-
2302	L Y V S P S M P E N L	1251.6	-
2303	L Y R S P S A P E N L	1248.4	-
2304	L Y R S P S R P E N L	1333.5	-
2305	L Y R S P S N P E N L	1291.5	-
2306	L Y R S P S D P E N L	1292.4	-
2307	L Y R S P S C P E N L	1280.5	-
2308	L Y R S P S Q P E N L	1305.5	-
2309	L Y R S P S E P E N L	1306.4	-
2310	L Y R S P S G P E N L	1234.4	-
2311	L Y R S P S H P E N L	1314.5	-
2312	L Y R S P S I P E N L	1290.5	-
2313	L Y R S P S L P E N L	1290.5	-
2314	L Y R S P S K P E N L	1305.5	-

2315	L Y R S P S M P E N L	1308.6	-
2316	L Y R S P S F P E N L	1324.5	-
2317	L Y R S P S P P E N L	1274.5	-
2318	L Y R S P S S P E N L	1265.2	-
2319	L Y R S P S T P E N L	1278.4	-
2320	L Y R S P S W P E N L	1363.6	-
2321	L Y R S P S Y P E N L	1340.5	-
2322	L Y R S P S V P E N L	1276.5	-
2323	L Y R S P S M A E N L	1282.5	-
2324	L Y R S P S M R E N L	1367.6	-
2325	L Y R S P S M N E N L	1325.6	-
2326	L Y R S P S M D E N L	1326.5	-
2327	L Y R S P S M C E N L	1314.6	-
2328	L Y R S P S M Q E N L	1339.6	-
2329	L Y R S P S M E E N L	1340.5	-
2330	L Y R S P S M G E N L	1268.5	-
2331	L Y R S P S M H E N L	1348.6	-
2332	L Y R S P S M I E N L	1324.6	-
2333	L Y R S P S M L E N L	1324.6	-
2334	L Y R S P S M K E N L	1339.6	-
2335	L Y R S P S M M E N L	1342.7	-
2336	L Y R S P S M F E N L	1358.6	-
2337	L Y R S P S M P E N L	1308.6	-
2338	L Y R S P S M S E N L	1299.3	-
2339	L Y R S P S M T E N L	1312.5	-
2340	L Y R S P S M W E N L	1397.7	-
2341	L Y R S P S M Y E N L	1374.6	-
2342	L Y R S P S M V E N L	1310.6	+

Example 3: **G2 abrogating peptides of the invention**

The following example describes studies which identified exemplary G2 checkpoint-abrogating peptides of the invention. The following peptides of the invention were synthesized directly on membranes and tested in *in vitro* phosphorylation ("kination" assays, as described above.

Table 2

PEPTIDE	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}
AAA	L	A	R	S	A	S	M	P	E	A	L
RANDOMII	R	Y	S	L	P	P	E	L	S	N	M
S216A	L	Y	R	S	P	A	M	P	E	N	L
S216P	L	Y	R	S	P	S	M	P	E	N	L
YPN		Y	G	G	P	G	G	G	G	N	
YG7N		Y	G	G	G	G	G	G	G	N	
YG6N		Y	G	G	G	G	G	G		N	
YG5N		Y	G	G	G	G	G			N	
YPN		Y			P					N	
RPL			R					P			L
YGN		Y			G					N	

These peptides were tested in *in vitro* kination reactions. The oligopeptides were used as phosphorylation substrates; added kinases are involved in the cell cycle G2 checkpoint. Thus, a substance that inhibits the kination reaction can be a cell cycle G2 checkpoint abrogator. For the detection of the phosphorylation status of substrates in this screening method, isotope-labeled ATP and anti-phospho-peptides antibody can be used.

hChk1; hChk1 fusion proteins (MBP-peptide, GST-peptide), HuCds1/Chk2; HuCds1/Chk2 fusion proteins (MBP-peptide, GST-peptide); or, the cell extract from DNA damaged cells, can be used as the kinases in the screening assay.

The oligopeptides tested as substrates are Y X_2 X_3 P S X_6 X_7 X_8 N (X_2 through X_9, respectively; the first position (X_1)"Y" in this abbreviated nine residue motif corresponds to position X_2 in the eleven residue motif, described above) and variations thereof wherein amino acid residues at positions 2 (X_2) and position 3 (X_3) are Gly, Leu, Ser, or Arg; and the amino acid residue at position 6 through 8 are Gly, Leu, Ser, Met, Pro or Glu. Other tested oligopeptides sequence variations have amino acid residues at position 2 as Gly, Leu, Ser, or Arg; amino acid residues at position 3 as Gly, Leu or Ser; amino acid residues at position 6 as Gly, Met, Pro or Glu; amino acid residues at position 7 as Gly, Leu, or Pro; and, amino acid residues at position 8 as Gly, Met, Ser or Glu. In another variation the residue at

position 2 was Arg; position 3 was Ser; position 6 was Met; position 7 was Pro; and, position 8 was Glu.

The cells with the deficient cell cycle G1 checkpoint (such as a human leukemia-derived cell line Jurkat) were treated with a DNA damaging treatment. As the DNA damaging treatment, the cells were treated with bleomycin or other anti-cancer drugs. These drugs were added to the cell culture medium. Alternatively, the cells were irradiated with gamma irradiation. Peptides were added to these cells and the amount of DNA was determined some 10 to 48 hours after the DNA damage. The harvested cells were re-suspended with the solution that includes propidium iodide, RNase and NP-40 and analyzed by flow cytometer. If the oligopeptide "candidate substance" induces cells not to accumulate DNA at G2/M by this analysis, the result is positive and the substance potentially abrogated G2/M checkpoint.

Other screening methods can be used to identify selective inhibitors of the G2 cell cycle checkpoint. For, the cells are simultaneously treated with an oligopeptide "candidate phosphorylation substrate" and an M phase checkpoint activator, such as colchicine or nocodazol. The DNA content of the cells are analyzed some 10 to 48 hours after the treatment as described above. The candidates that do not disturb the accumulation of the cells at G2/M will be the selected G2 checkpoint abrogators in this screening method.

In one embodiment, G2 checkpoint abrogators at positions 2 and 3 the have amino acid residues Gly, Leu, Ser or Arg, and at position 5 to 8 are amino acid residues Ser, Gly, Met, Pro or Glu.

In one embodiment of the invention the compositions are enhancers or augmenters of a DNA damaging anti-cancer treatment. By treating cancer cells simultaneously or sequentially with an anti-cancer treatment and a G2 checkpoint inhibiting composition of the invention, one can effectively kill the cancer cells. Since the most human cancer cells do not have an intact G1 checkpoint, the abrogation of the G2 checkpoint by a G2 checkpoint inhibiting composition of the invention will effectively kill the cancer cells that are treated with a DNA damaging method. The compositions of the invention can be directly used as a drug (e.g., a pharmaceutical compositions) or these oligopeptides could be expressed recombinantly *in vivo*, e.g., from a virus vector or other expression vector, e.g., a plasmid, as an *in vivo* gene therapy.

Jurkat cells were cultured in 10% fetal calf serum with a medium (RPMI 1640) at 37°C/5% CO_2 with: bleomycin at 20 μg/ml; bleomycin at 20 μg/ml and the peptide "4aa" (amino acid sequence is GGSPSM); bleomycin at 20 μg/ml and the peptide AAA (Table 1); bleomycin at 20 μg/ml and the peptide YNP (Table 1). The amount of DNA was analyzed at 0, 6, 12, 24 hours after the addition of ten microgram of bleomycin with or without the oligopeptides "4aa," "YNP" and "AAA." The DNA quantity was analyzed by a flow cytometer (FACS) after the addition of a solution comprising propidium iodide, RNase and NP-40.

The results are shown in Figure 6. The left panels are actual results of flow cytometer (FACS) analysis. The right panel indicates the population of cells in each of the cell cycle phases (sub G1, G1, S, and G2/M). The results indicated that YNP peptide abrogated the G2 checkpoint because the cells do not accumulate at G2/M phases.

In another experiment, an M phase checkpoint activator, colchicine, was used instead of bleomycin: colchicine at 2.5 μg/ml; colchicine at 2.5 μg/ml and the peptide "4aa"; colchicine at 2.5 μg/ml and the peptide AAA (Table 1); colchicine at 2.5 μg/ml and the peptide YNP (Table 1), and no treatment. The results are shown in Figure 7. None of the above tested oligopeptides (Table 1), including, YPN, affected the accumulation of the colchicine-treated cells at the G2/M phase. These data indicated that YPN specifically abrogated the cell cycle at the G2 checkpoint.

Peptides which were tested and the results of these experiments are further summarized in Figures 8 and 9.

<u>Example 4</u>: Peptides of the invention sensitize cancer cells in *in vivo* animal model

The following example describes studies in an art-accepted animal model which demonstrated that exemplary peptides of the invention are effective agents for selectively sensitizing cancer cells to DNA damaging agents. In particular, nude mouse studies demonstrated the *in vivo* efficacy of the compositions and methods of the invention.

Human colon cancer cell line SW620 were injected subcutaneously into 3 week old Balb/c nude mouse ($1x10^8$ cells per mouse). Some two weeks after the injection, the established subcutaneous tumors of diameter 2 to 4 mm were resected and transplanted to syngeneic mice. One week after the transplantation, the injection of cisplatin (CDDP) and peptides (TAT-control and TAT-S216, see Table 1) was started. The peptides were in the

form of recombinant fusion proteins, with TAT being the protein transduction domain having the sequence YGRKKRRQRRR.

Cisplatin (CDDP) at 6 mg/kg was injected once a week into peritoneum. Peptides (at 100 nM) were injected into tumor twice a week. Relative tumor weights were assessed at 3 and 5 weeks. The results are shown in Figure 10, upper panel. Similar experiments were performed with 5-FU instead of cisplatin. The results are shown in Figure 8, lower panel. As shown in Figure 10, the S216-containing fusion protein effectively sensitized the cancer cells to a DNA damaging agent administered to the animal *in vivo*.

Similar experiments were performed with cisplatin (CDDP) and another exemplary peptide of the invention, "random II" or "R-II" (see Table 1). As with S216, RII peptide was in the form of a recombinant fusion protein with TAT. The relative volume of the transplanted subcutaneous tumor with or without cisplatin ("CDDP"), CDDP plus DMSO, CDDP plus TAT-FLAG or CDDP plus TAT-Random II peptide was determined. As shown in Figure 11, the R-II containing fusion protein effectively sensitized the cancer cells to a DNA damaging agent administered to the animal *in vivo*.

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. An isolated or recombinant polypeptide comprising the amino acid sequence:

$$X_1\ X_2\ X_3\ X_4\ X_5\ X_6\ X_7\ X_8\ X_9\ X_{10}\ X_{11}$$

wherein X1 is L, F, W, M, R, I, V, Y, K, or absent,

X2 is Y, F, A, W, S or T,

X3 is any amino acid,

X4 is any amino acid,

X5 is any amino acid,

X6 is S, A, N, H or P,

X7 is any amino acid,

X8 is any amino acid,

X9 is any amino acid or absent,

X10 is N, G, L, S, M, P, N, A or absent, and

X11 is L or absent,

wherein the polypeptide when administered to or expressed in a cell disrupts the G2 cell cycle arrest checkpoint.

2. The isolated or recombinant polypeptide of claim 1, wherein X_1 is L, F, W, M, R or absent.

3. The isolated or recombinant polypeptide of claim 2, wherein X_1 is L, F or W.

4. The isolated or recombinant polypeptide of claim 1, wherein X_2 is Y, F, A.

5. The isolated or recombinant polypeptide of claim 1, wherein X_3 is R, T, S, H, D, G, A, L, K, A, N, Q or P.

6. The isolated or recombinant polypeptide of claim 5, wherein X_3 is R, T, S, H, D, G, A or L.

7. The isolated or recombinant polypeptide of claim 6, wherein X_3 is R, T, S or H.

8. The isolated or recombinant polypeptide of claim 1, wherein X_4 is S, T, G, A, L, R, I, M, V, P.

9. The isolated or recombinant polypeptide of claim 8, wherein X_4 is S, T, G, A, L, R .

10. The isolated or recombinant polypeptide of claim 9, wherein X_4 is S.

11. The isolated or recombinant polypeptide of claim 1, wherein X_5 is P, A, G, S or T.

12. The isolated or recombinant polypeptide of claim 1, wherein X_5 is P.

13. The isolated or recombinant polypeptide of claim 1, wherein X_6 is S, N, H, P, A, G or T.

14. The isolated or recombinant polypeptide of claim 13, wherein X_6 is S, N or H.

15. The isolated or recombinant polypeptide of claim 14, wherein X_6 is S.

16. The isolated or recombinant polypeptide of claim 1, wherein X_7 is M, F, Y, D, E, N, Q, H, G, I, L, V, A, P, N or W.

17. The isolated or recombinant polypeptide of claim 16, wherein X_7 is M, F, Y, D, E, N, Q or H.

18. The isolated or recombinant polypeptide of claim 17, wherein X_7 is M, F, Y, Q or H.

19. The isolated or recombinant polypeptide of claim 1, wherein X_8 is P, F, Y, W, L, G, M, D, E, N, Q, H, I, V, A or P.

20. The isolated or recombinant polypeptide of claim 19, wherein X_8 is P, F, Y or W.

21. The isolated or recombinant polypeptide of claim 20, wherein X_8 is Y.

22. The isolated or recombinant polypeptide of claim 1, wherein X_9 is E, G, L, S, M, P, N, D, A, T, P or absent.

23. The isolated or recombinant polypeptide of claim 1, wherein X_{10} is absent.

24. The isolated or recombinant polypeptide of claim 1, wherein X_{11} is absent.

25. The isolated or recombinant polypeptide of claim 1, wherein X_2 is Y, X_5 is P, and X_{10} is N.

26. The isolated or recombinant polypeptide of claim 1, wherein X_3 is R, X_8 is P, and X_{11} is L.

27. The isolated or recombinant polypeptide of claim 1, wherein X_4 is S, X_5 is P, X_6 is S, X_9 is E, X_{10} is N and X_{11} is L.

28. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises Y G G P G G G G N.

29. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises R Y S L P P E L S N M.

30. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises L A R S A S M P E A L.

31. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises L Y R S P S M P E N L.

32. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises L Y R S P A M P E N L.

33. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises W Y R S P S F Y E N L.

34. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises W Y R S P S Y Y E N L.

35. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises W Y R S P S Y Y.

36. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises L Y R S P S Y P E N L, L Y R S P S Y F E N L, L Y R S P S Y Y E N L, or L Y R S P S Y W E N L.

37. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises L Y R S P S N P E N L, L Y R S P S N F E N L, L Y R S P S N Y E N L, or L Y R S P S N W E N L.

38. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises L Y R S P S H P E N L, L Y R S P S H F E N L, L Y R S P S H Y E N L, L Y R S P S H W E N L, L Y S S P S M P E N L, L Y S S P S M F E N L, L Y S S P S M Y E N L, L Y S S P S M W E N L, L Y S S P S F P E N L, L Y S S P S F P E N L,, L Y S S P S F F E N L, L Y S S P S F Y E N L, L Y S S P S F W E N L, L Y S S P S Y P E N L, L Y S S P S Y F E N L, L Y S S P S Y Y E N L, or L Y S S P S Y W E N L.

39. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises L Y S S P S Q P E N L, L Y S S P S Q W E N L, L Y S S P S H P E N L, L Y S S P S H F E N L, L Y S S P S H Y E N L, L Y S S P S H W E N L, L Y T S P S M P E N L, L Y T S P S M F E N L, L Y T S P S M Y E N L, L Y T S P S M W E N L, L Y T S P S F P E N L, L Y T S P S F F E N L, L Y T S P S F Y E N L, L Y T S P S F W E N L, L Y T S P S Y P E N L, L Y T S P S Y F E N L, L Y T S P S Y Y E N L, or L Y T S P S Y W E N L.

40. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises L Y T S P S N P E N L, L Y T S P S N F E N L, L Y T S P S N Y E N L or L Y T S P S N W E N L.

41. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises L Y T S P S H P E N L, L Y T S P S H F E N L, L Y T S P S H Y E N L or L Y T S P S H W E N L.

42. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises L Y H S P S Y P E N L, L Y H S P S Y F E N L, L Y H S P S Y Y E N L or L Y H S P S Y W E N L.

43. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises L F T S P S Y P E N L, L F T S P S Y F E N L, L F T S P S Y Y E N L or L F T S P S Y W E N L.

44. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises F Y S S P S H P E N L, F Y S S P S H F E N L, F Y S S P S H Y E N L, F Y S S P S H W E N L, F Y T S P S M P E N L, F Y T S P S M F E N L, F Y T S P S M Y E N L, F Y T S P S M W E N L, F Y T S P S F P E N L, F Y T S P S F F E N L, F Y T S P S F Y E N L, F Y T S P S F W E N L, F Y T S P S Y P E N L, F Y T S P S Y F E N L, F Y T S P S Y Y E N L or F Y T S P S Y W E N L.

45. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises W Y R S P S M P E N L, W Y R S P S M F E N L, W Y R S P S M Y E N L, W Y R S P S M W E N L, W Y R S P S F P E N L, W Y R S P S F F E N L, W Y R S P S F Y E N L, W Y R S P S F W E N L, W Y R S P S Y P E N L, W Y R S P S Y F E N L, W Y R S P S Y Y E N L or W Y R S P S Y W E N L.

46. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises W Y T S P S M P E N L, W Y T S P S M F E N L, W Y T S P S M Y E N L, W Y T S P S M W E N L, W Y T S P S F P E N L, W Y T S P S F F E N L, W Y T S P S F Y E N L, W Y T S P S F W E N L, W Y T S P S Y P E N L, W Y T S P S Y F E N L, W Y T S P S Y Y E N L or W Y T S P S Y W E N L.

47. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises W Y T S P S H P E N L, W Y T S P S H F E N L, W Y T S P S H Y E N L or W Y T S P S H W E N L.

48. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises L K R S P S M P E N L, L Y I S P S M P E N L or L Y R S P S M V E N L.

49. The isolated or recombinant polypeptide of claim 1, wherein the cell is a mammalian cell.

50. The isolated or recombinant polypeptide of claim 49, wherein the cell is a human cell.

51. The isolated or recombinant polypeptide of claim 1, further comprising a cell membrane permeant.

52. The isolated or recombinant polypeptide of claim 51, wherein the cell membrane permeant comprises a polypeptide.

53. The isolated or recombinant polypeptide of claim 52, wherein the polypeptide comprises a TAT protein transduction domain.

54. The isolated or recombinant polypeptide of claim 53, wherein the TAT protein transduction domain is Y G R K K R R Q R R R.

55. The isolated or recombinant polypeptide of claim 51, wherein the cell membrane permeant comprises a lipid.

56. The isolated or recombinant polypeptide of claim 55, wherein the cell membrane permeant comprises a liposome.

57. A chimeric polypeptide comprising a first domain comprising a polypeptide as set forth in claim 1 and a second domain comprising a cell membrane

permeant, wherein the polypeptide when administered to or expressed in a cell disrupts the G2 cell cycle arrest checkpoint.

58. The chimeric polypeptide of claim 57, wherein the polypeptide is a recombinant fusion protein.

59. An isolated or recombinant nucleic acid encoding a polypeptide as set forth in claim 1 or claim 57, wherein the polypeptide when administered to or expressed in a cell disrupts the G2 cell cycle arrest checkpoint.

60. An expression vector comprising a nucleic acid encoding a polypeptide as set forth in claim 1 or claim 57, wherein the polypeptide when administered to or expressed in a cell disrupts the G2 cell cycle arrest checkpoint.

61. A cell comprising a nucleic acid encoding a polypeptide as set forth in claim 1 or claim 57, wherein the polypeptide when administered to or expressed in a cell disrupts the G2 cell cycle arrest checkpoint.

62. The cell of claim 61, wherein the cell is a bacterial, a yeast, an insect, or a mammalian cell.

63. A pharmaceutical composition comprising a

a polypeptide as set forth in claim 1 or claim 57, wherein the polypeptide when administered to or expressed in a cell disrupts the G2 cell cycle arrest checkpoint,

a nucleic acid encoding a polypeptide as set forth in claim 1 or claim 57, wherein the polypeptide when administered to or expressed in a cell disrupts the G2 cell cycle arrest checkpoint,

an expression vector comprising a nucleic acid encoding a polypeptide as set forth in claim 1 or claim 57, wherein the polypeptide when administered to or expressed in a cell disrupts the G2 cell cycle arrest checkpoint, or

a cell comprising a nucleic acid encoding a polypeptide as set forth in claim 1 or claim 57, wherein the polypeptide when administered to or expressed in a cell disrupts the G2 cell cycle arrest checkpoint; and,

a pharmaceutically acceptable excipient.

64. The pharmaceutical composition of claim 63 comprising a liposome.

65. A method for inhibiting a the activity of a Chk1 kinase or a Chk2 kinase comprising contacting the kinase with a polypeptide as set forth in claim 1 or claim 57 or a pharmaceutical composition as set forth in claim 63, in an amount sufficient to inhibit the activity of the Chk1 or Chk2 kinase.

66. A method for disrupting a cell G2 cell cycle arrest checkpoint comprising contacting the cell with a polypeptide as set forth in claim 1 or claim 57 or a pharmaceutical composition as set forth in claim 63, in an amount sufficient to disrupt the G2 cell cycle arrest checkpoint.

67. A method for sensitizing a cell to a DNA damaging agent comprising contacting the cell with a polypeptide as set forth in claim 1 or claim 57 or a pharmaceutical composition as set forth in claim 63, in an amount sufficient to disrupt the G2 cell cycle arrest checkpoint, thereby sensitizing the cell to the DNA damaging agent.

68. The method of claim 67, wherein the cell is a human cell.

69. The method of claim 67, wherein the cell is a cancer cell.

70. A method for selectively sensitizing a cell with an impaired G1 cell cycle arrest checkpoint to a DNA damaging agent comprising contacting the cell with a polypeptide as set forth in claim 1 or claim 57 or a pharmaceutical composition as set forth in claim 63, in an amount sufficient to disrupt the G2 cell cycle arrest checkpoint, thereby sensitizing the cell to the DNA damaging agent.

71. The method of claim 70, wherein the cell is a cancer cell.

72. A method for inducing apoptosis in a cancer cell in an individual comprising a administering a polypeptide as set forth in claim 1 or claim 57 or a pharmaceutical composition as set forth in claim 63, in an amount sufficient to disrupt the G2 cell cycle arrest checkpoint in the cancer cell, thereby sensitizing the cancer cell to a DNA damaging agent, and administering a DNA damaging agent.

73. The method of claim 72, wherein the DNA damaging agent is 5-fluorouracil (5-FU), rebeccamycin, adriamycin, bleomycin, cisplatin, hyperthermia, UV irradiation or gamma-irradiation.

74. A method for screening for compounds capable of modulating the activity of a Chk1 kinase or a Chk2 kinase comprising the following steps

(a) providing a test compound;

(b) providing a Chk1 kinase or a Chk2 kinase;

(c) providing a polypeptide as set forth in claim 1 or claim 57, wherein the polypeptide binds to the Chk1 kinase or the Chk2 kinase; and

(d) contacting the test compound with the kinase and the polypeptide and measuring the ability of the test compound to prevent binding of the polypeptide to the kinase.

75. A method for screening for compounds capable of modulating the activity of a Chk1 kinase or a Chk2 kinase comprising the following steps

(a) providing a test compound;

(b) providing a Chk1 kinase or a Chk2 kinase;

(c) providing a polypeptide as set forth in claim 1 or claim 57, wherein the polypeptide is phosphorylated by the Chk1 kinase or the Chk2 kinase; and

(d) contacting the test compound with the kinase and the polypeptide and measuring the ability of the test compound to inhibit or abrogate phosphorylation of the polypeptide by the kinase.

76. The method of claim 75 further comprising providing a full length human Cdc25C.

77. The method of claim 75, wherein the polypeptide of step (c) comprises amino acid residue serine 216 of human Cdc25C.

78. The method of claim 77, wherein the polypeptide is a peptide comprising from about amino acid residue 200 to about amino acid residue 250 of human Cdc25C.

79. The method of claim 74 or claim 75, wherein the polypeptide of step (c) further comprises glutathione-S-transferase.

80. The method of claim 74 or claim 75, wherein the polypeptide of step (c) is immobilized.

81. A method for screening for compounds capable of specifically inhibiting or abrogating the G2 cell cycle arrest checkpoint comprising the following steps

(a) providing a test compound and a polypeptide as set forth in claim 1 or claim 57;

(b) providing a G1 checkpoint impaired cell;

(c) contacting the cell of step (b) with the test compound or the polypeptide of step (a) and a DNA damaging treatment or an M phase checkpoint activator; and

(d) measuring the amount of DNA in the cells after the contacting of step (c) to determine if the test compound has inhibited or abrogated the G2 cell cycle arrest checkpoint, wherein the polypeptide of step (a) acts as a G2-checkpoint-inhibiting positive control.

82. The method of claim 81, wherein the amount of DNA is measured using propidium iodide and FACS analysis.

83. The method of claim 81, wherein the amount of DNA is measured after about 10 to about 72 hours after the contacting of step (c).

84. The method of claim 81, wherein the cell is contacted with an M phase checkpoint activator and a test compound or a polypeptide of step (a), wherein a test compound that has not inhibited or abrogated the arrest at the M phase checkpoint of the cell cycle after contacting the cell with an M phase activator is a specific inhibitor of the G2 cell cycle arrest checkpoint.

85. The method of claim 84, wherein the M phase checkpoint activator is colchicine or nocodazole.

86. The method of claim 81, wherein the DNA damaging treatment is 5-fluorouracil (5-FU), rebeccamycin, adriamycin, bleomycin, cisplatin, hyperthermia, UV irradiation or gamma-irradiation.

Fig. 1

A

TAT-S216 — TAT: YGRKKRRQRRR — S216: LYRSP(S)MPENL

TAT-S216A — TAT: YGRKKRRQRRR — S216A: LYRSP(A)MPENL

TAT-Control — TAT: YGRKKRRQRRR — GGRSPAMPE

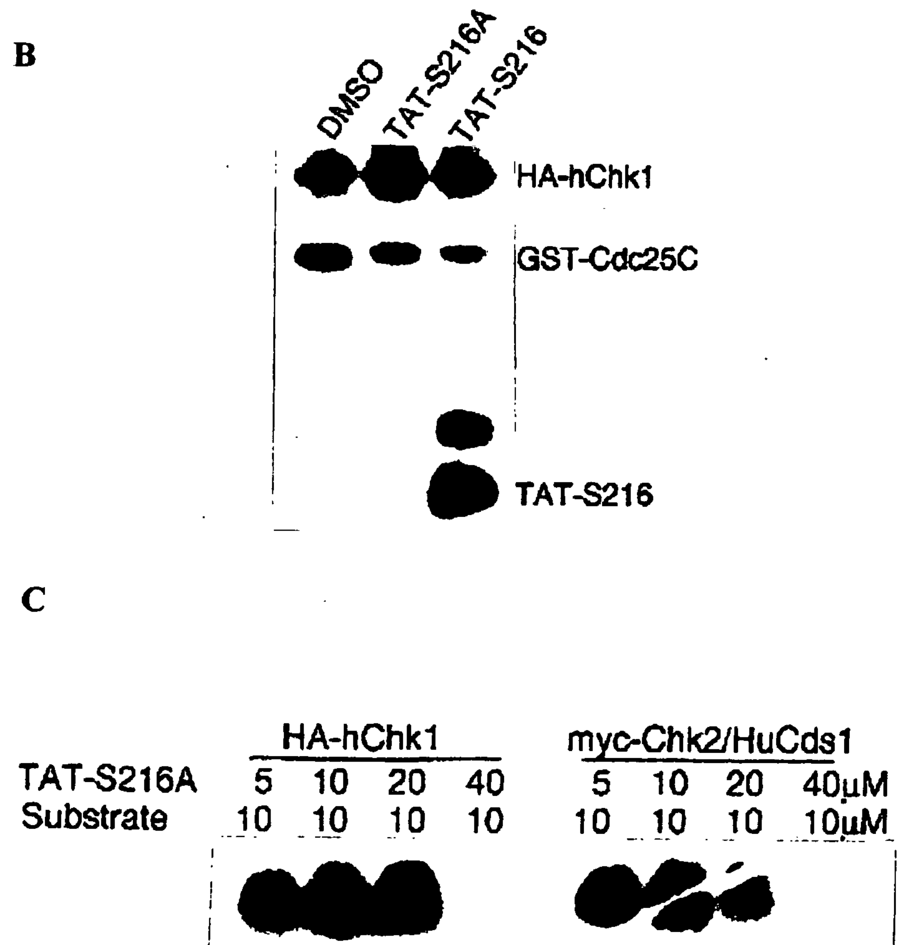
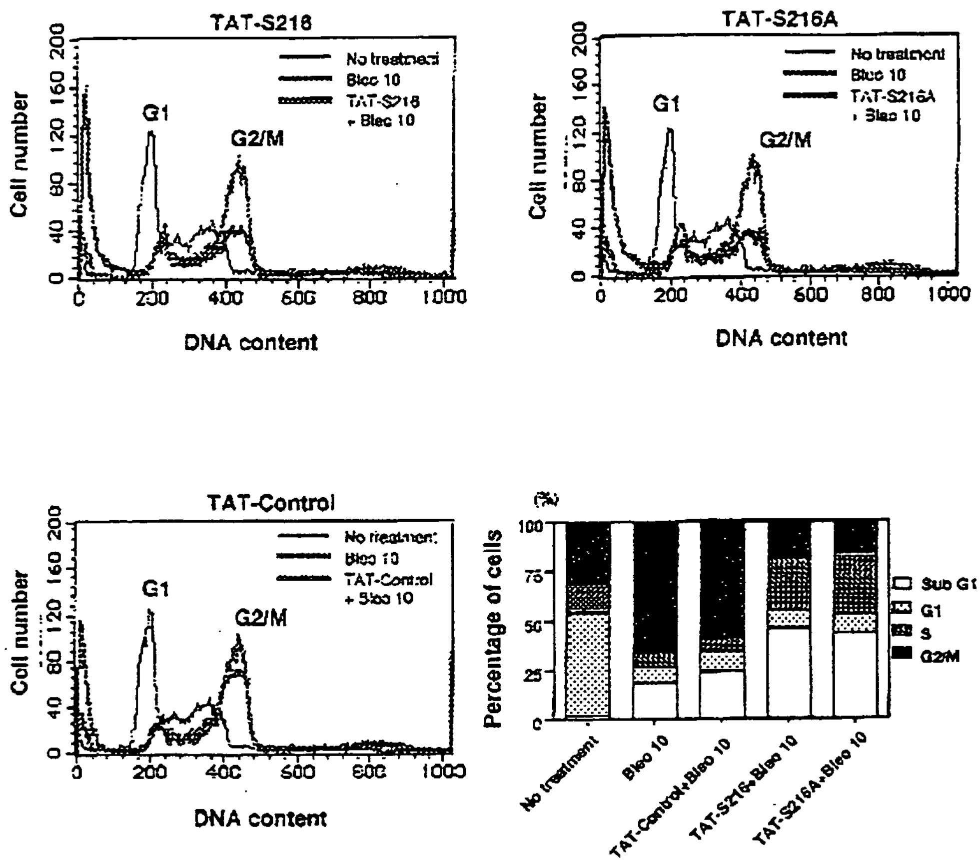


Fig. 2

A


TAT-S216
No treatment
Bleo 10
TAT-S216 + Bleo 10
G1
G2/M
Cell number
DNA content
TAT-S216A
No treatment
Bleo 10
TAT-S216A + Bleo 10
TAT-Control
No treatment
Bleo 10
TAT-Control + Bleo 10
(%)
Percentage of cells
Sub G1
G1
S
G2/M
No treatment
Bleo 10
TAT-Control+Bleo 10
TAT-S216+Bleo 10
TAT-S216A+Bleo 10

B


No treatment
HU
Bleo
Colchicine
Bleo + TAT-S216A
Bleo + TAT-S216

C

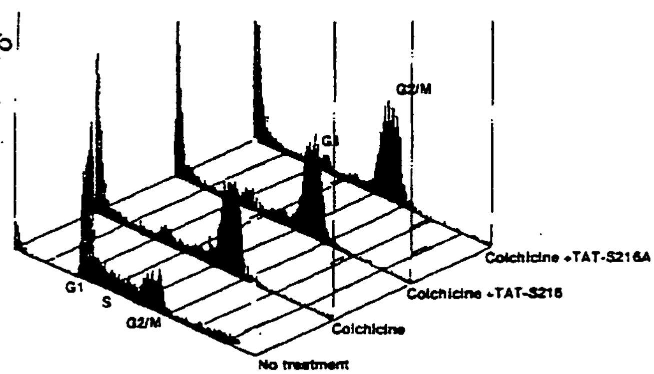

G2/M
G1
S
Colchicine +TAT-S216A
Colchicine +TAT-S216
Colchicine
No treatment

Fig. 3

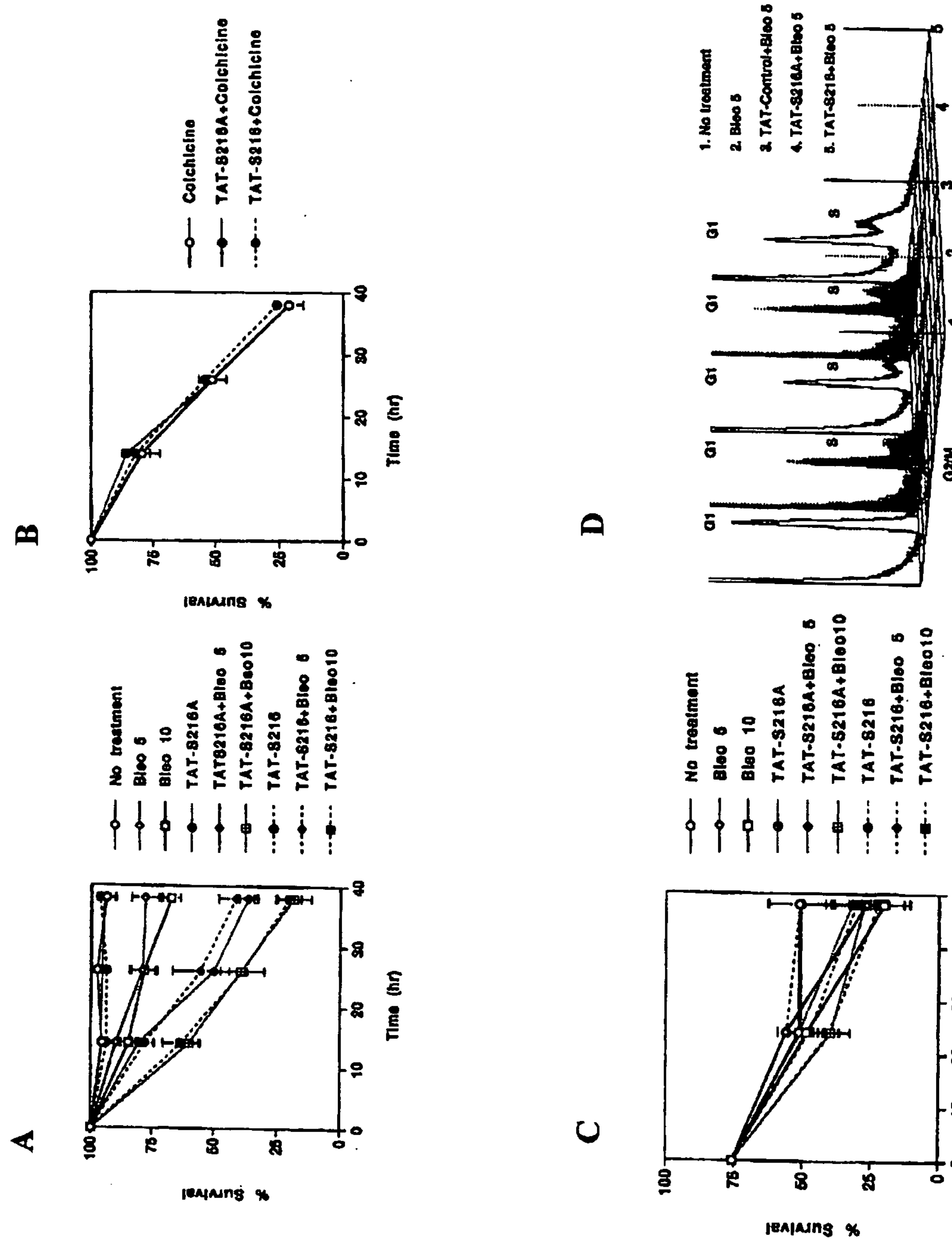

A
B
C
D
% Survival
Time (hr)
No treatment
Bleo 5
Bleo 10
TAT-S216A
TATS216A+Bleo 5
TAT-S216A+Bleo10
TAT-S216
TAT-S216+Bleo 5
TAT-S216+Bleo10
Colchicine
TAT-S216A+Colchicine
TAT-S216+Colchicine
1. No treatment
2. Bleo 5
3. TAT-Control+Bleo 5
4. TAT-S216A+Bleo 5
5. TAT-S216+Bleo 5
G1
S
G2/M

Fig. 4

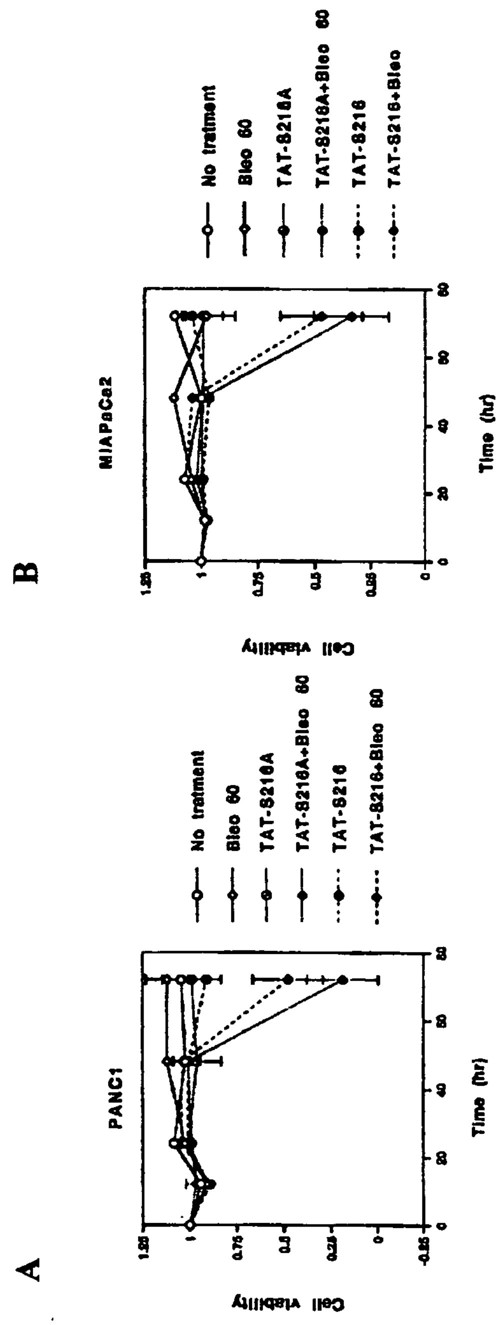

A
PANC1
Cell viability
Time (hr)
No tratment
Bleo 60
TAT-S216A
TAT-S216A+Bleo 60
TAT-S216
TAT-S216+Bleo 60
B
MIAPaCa2
Cell viability
Time (hr)
No tratment
Bleo 60
TAT-S216A
TAT-S216A+Bleo 60
TAT-S216
TAT-S216+Bleo

Fig. 5

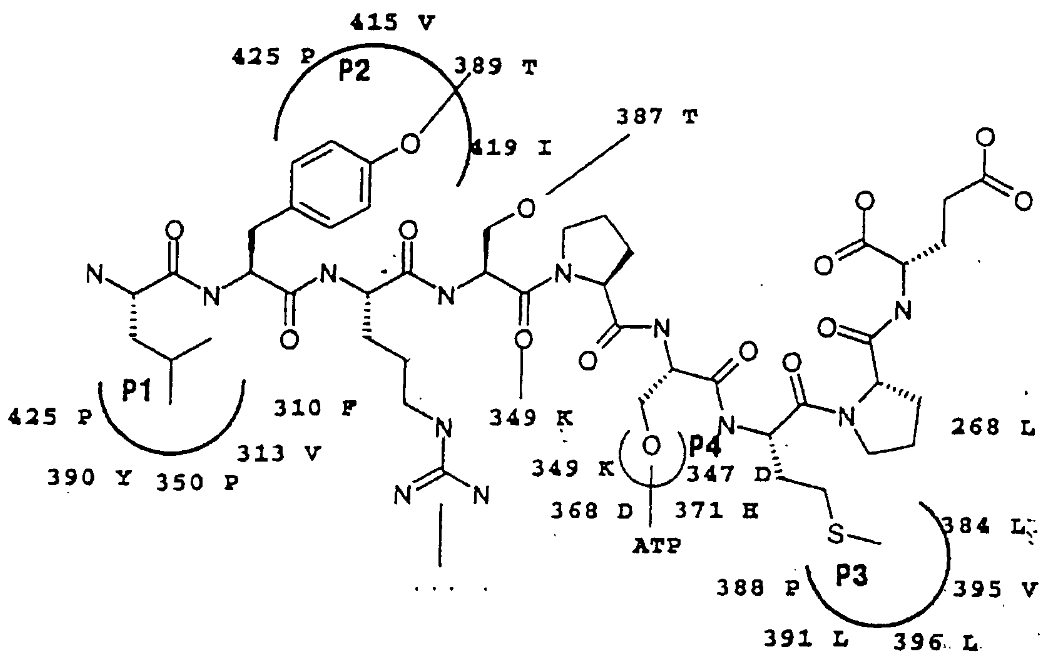
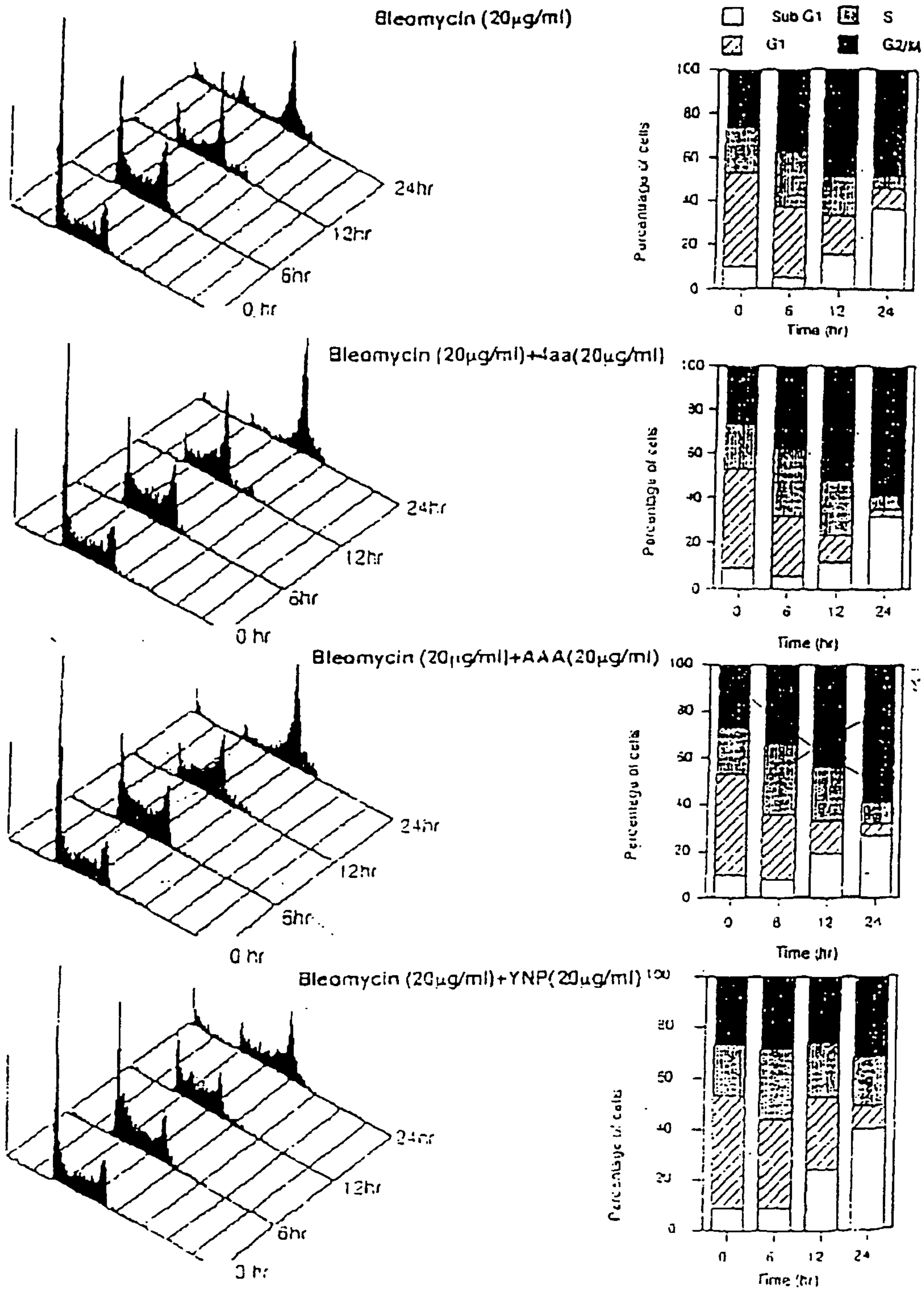
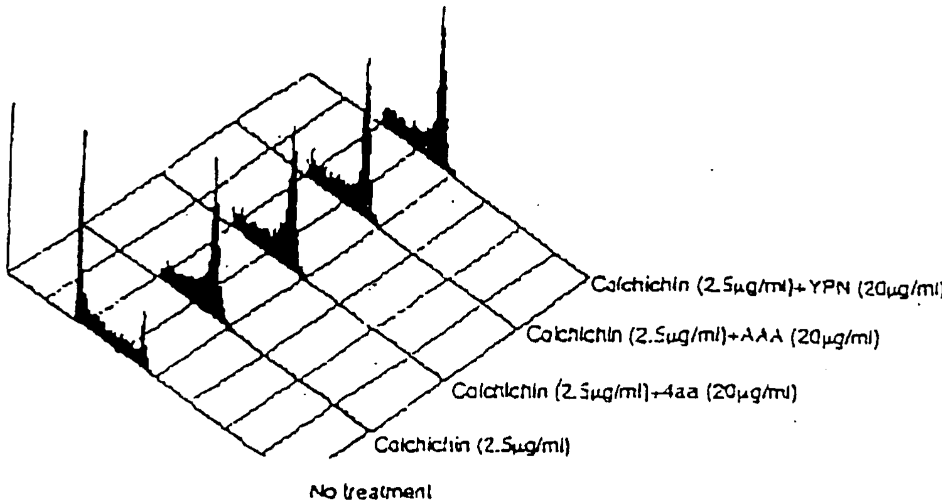

415 V
425 P
P2
389 T
387 T
419 I
P1
425 P
310 F
313 V
390 Y
350 P
349 K
349 K
P4
347 D
368 D
371 H
ATP
268 L
384 L
P3
388 P
395 V
391 L
396 L

Fig. 6


Bleomycin (20µg/ml)
Sub G1
S
G1
G2/M
Percentage of cells
Time (hr)
0 hr
6hr
12hr
24hr
Bleomycin (20µg/ml)+4aa(20µg/ml)
Bleomycin (20µg/ml)+AAA(20µg/ml)
Bleomycin (20µg/ml)+YNP(20µg/ml)


Colchichin (2.5μg/ml)+YPN (20μg/ml)
Colchichin (2.5μg/ml)+AAA (20μg/ml)
Colchichin (2.5μg/ml)+4aa (20μg/ml)
Colchichin (2.5μg/ml)
No treatment

【図4】

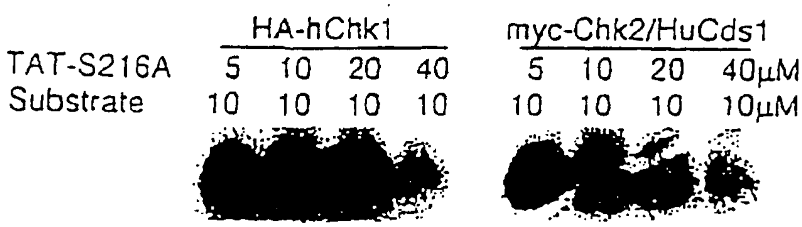

HA-hChk1
myc-Chk2/HuCds1
TAT-S216A 5 10 20 40 5 10 20 40μM
Substrate 10 10 10 10 10 10 10 10μM

Fig. 7

Name	Sequences
AAA	YGRKKRRQRRR LARSASMPEAL
YPN	YGRKKRRQRRR YGGPGGGGN
Random I	YGRKKRRQRRR YLSRSPPMNEL
Random II	YGRKKRRQRRR RYSLPPELSNM
S216A	YGRKKRRQRRR LYRSPAMPENL
S216P	YGRKKRRQRRR LYRSPSMPENL
SPAMPE	YGRKKRRQRRR GGRSPAMPE
SPAMPE	YGRKKRRQRRR GGSPAMP
RSPSMP	YGRKKRRQRRR GGRSPSMP
SPSMP	YGRKKRRQRRR GGSPSMP
SPAM	YGRKKRRQRRR GGSPAM
SPSM	YGRKKRRQRRR GGSPSM
YG7N	YGRKKRRQRRR YGGGGGGGN
YG6N	YGRKKRRQRRR YGGGGGGN
YG5N	YGRKKRRQRRR YGGGGGN
YXPXN	Tyr-NH(CH$_2$)4CO-Pro-NH(CH$_2$)10CO-Asn
YX10N	Tyr-NH(CH$_2$)10CO-Asn
YX4N	Tyr-NH(CH$_2$)4CO-Asn
TAT-HA	YGRKKRRQRRR YPYDVPDYA
TAT-FLAG	YGRKKRRQRRR GGDYKDDDDKG

Fig. 8

Fig. 9

SUMMARY G2 ABROGATION/Bleomycin

	10µM	20µM	40µM	80µM	160µM
No peptides	-	-	-	-	-
DMSO	-	-	-	-	-
FLAG	-	-	-	-	-
S216A	+	+	+	+	+
S216	+	+	+	+	+
Random II	+	+	+	+	+
YPN	-	+/-	+	+	+
YG7N	-	+/-	+	+	+
YG6N	-	+/-	+	+	+
YG5N	-	+/-	+	+	+
AAA	-	+/-	+	+	+
4aa	-	-	-	+/-	+

G2 ABROGATION/γ-radiarion

	10µM	20µM	40µM
No peptides	-	N.D.	-
DMSO	-	N.D.	-
FLAG	-	N.D.	-
S216A	-	N.D.	+
Random II	+/-	N.D.	+

G2 ABROGATION/UV

	10µM	20µM	40µM
No peptides	-	N.D.	-
DMSO	-	N.D.	-
FLAG	-	N.D.	-
S216A	-	N.D.	+
Random II	-	N.D.	+

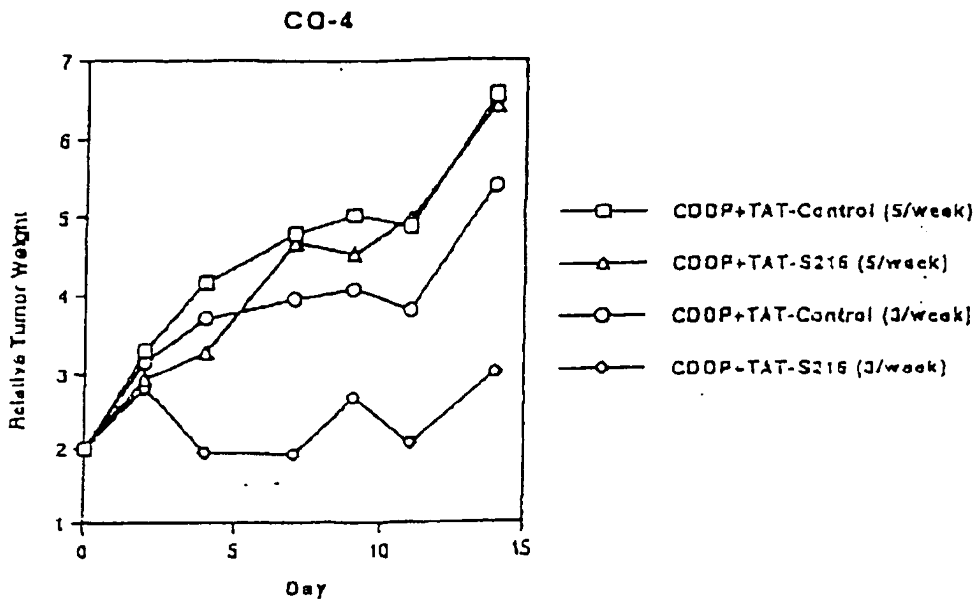
SUMMARY GstChk2 KINATION INHIBITION

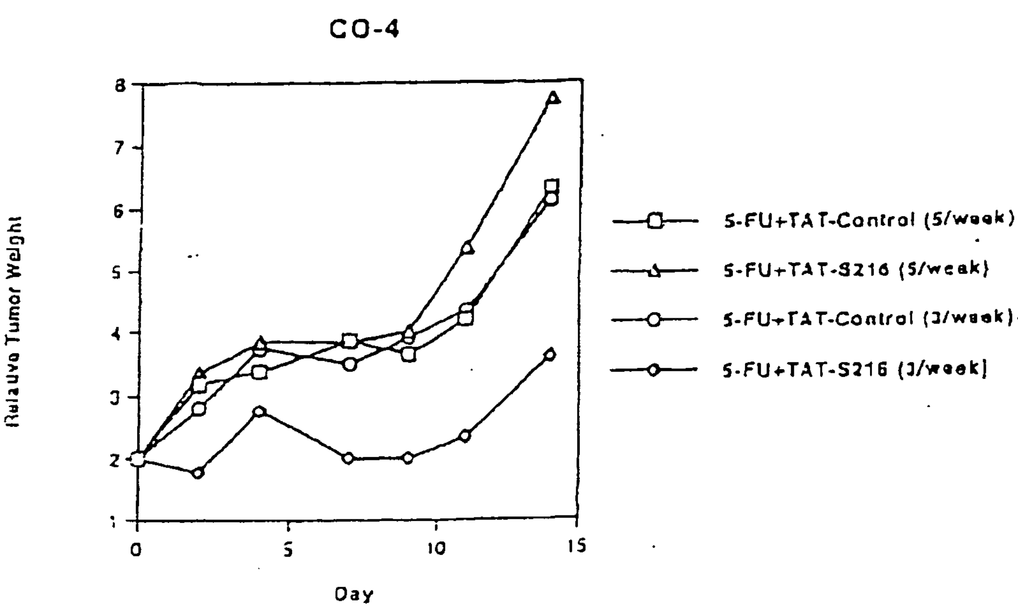
	10µM	20µM	40µM	80µM	160µM
No peptides	-	-	-	-	N.D.
DMSO	-	-	-	-	N.D.
FLAG	-	-	-	-	N.D.
S216A	+/-	+	++	++	N.D.
S216	+/-	+	++	++	N.D.
Random II	+/-	+	++	++	N.D.
YPN	+/-	+/-	+	+	N.D.
YG7N	+/-	+/-	+	+	N.D.
YG6N	+/-	+/-	+	++	N.D.
YG5N	+/-	+/-	+	+	N.D.
AAA	+/-	+/-	+	+	N.D.
4aa	-	-	-	-	N.D.

M ABROGATION/Cochicine

	10µM	20µM	40µM
No peptides	-	N.D.	-
DMSO	-	N.D.	-
FLAG	-	N.D.	-
S216A	-	N.D.	-
Random II	-	N.D.	-

Fig. 10


CO-4
Relative Tumor Weight
Day
CDDP+TAT-Control (5/week)
CDDP+TAT-S216 (5/week)
CDDP+TAT-Control (3/week)
CDDP+TAT-S216 (3/week)


CO-4
Relative Tumor Weight
Day
5-FU+TAT-Control (5/week)
5-FU+TAT-S216 (5/week)
5-FU+TAT-Control (3/week)
5-FU+TAT-S216 (3/week)

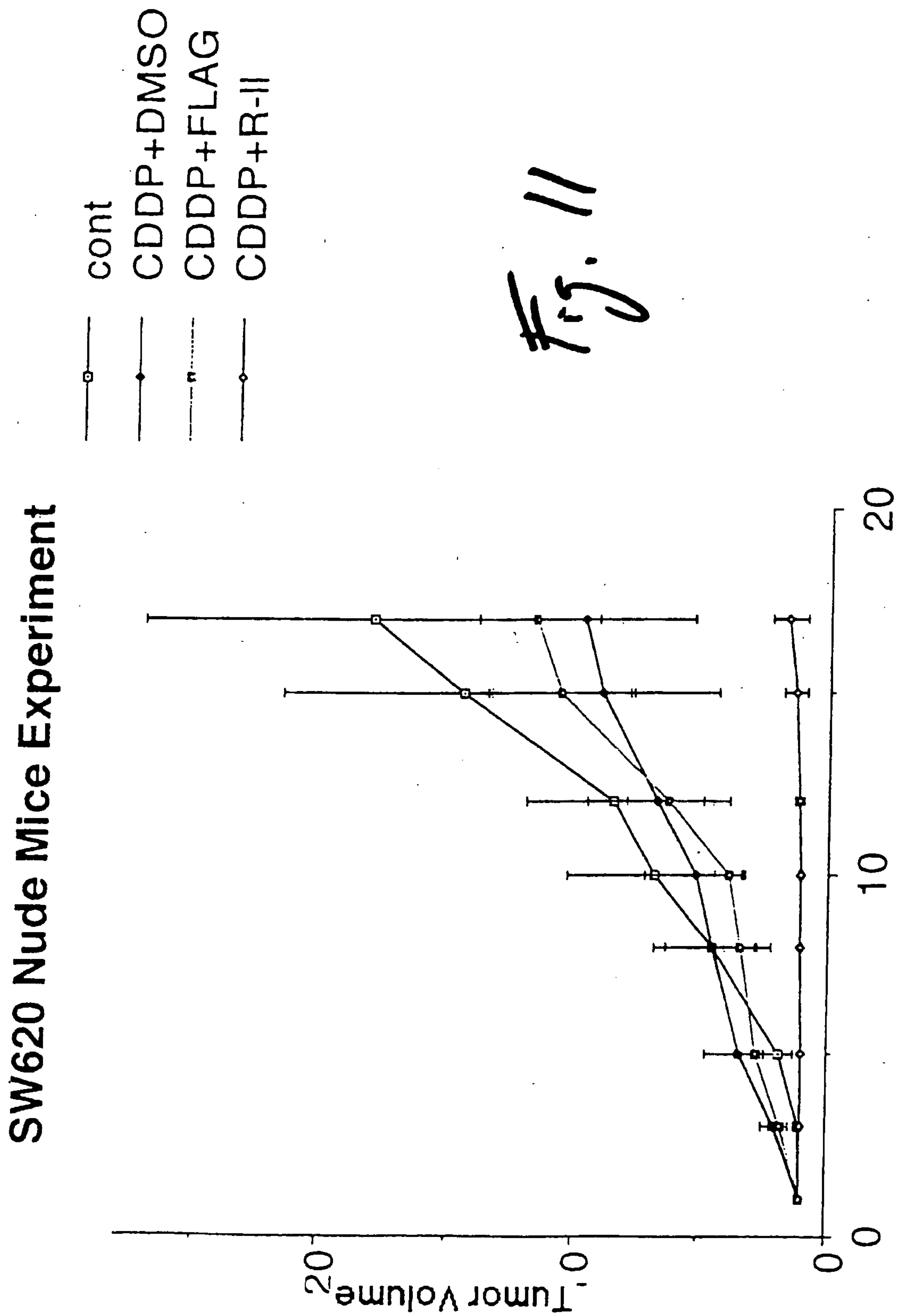

SW620 Nude Mice Experiment
cont
CDDP+DMSO
CDDP+FLAG
CDDP+R-II
Tumor Volume
20
0
day
0
10
20

Fig. 11

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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International Bureau

(43) International Publication Date
29 March 2001 (29.03.2001)

PCT

(10) International Publication Number
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(22) International Filing Date: 21 September 2000 (21.09.2000)

(25) Filing Language: English

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11/340322 30 November 1999 (30.11.1999) JP

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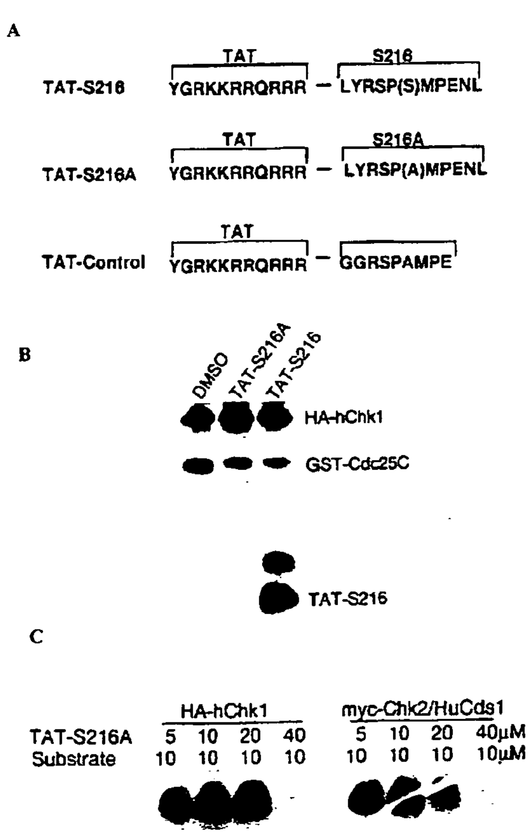
(74) Agent: **NISHIZAWA, Toshio**; 6F, Mani-Building, 37-10, Udagawa-cho, Shibuya-cho, Tokyo 150-0042 (JP).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European

[Continued on next page]

(54) Title: COMPOSITIONS AND METHODS FOR INHIBITING G2 CELL CYCLE ARREST AND SENSITIZING CELLS TO DNA DAMAGING AGENTS



(57) Abstract: The invention provides compositions and methods for inhibiting Chk1 and/or Chk2 kinases. Also provided are compositions and methods for inhibiting G2 cell arrest checkpoint, particularly in mammalian, e.g., human, cells. The compositions and methods of the invention are also used to treat disorders of cell growth, such as cancer. In particular, the invention provides methods for selectively sensitizing G1 checkpoint impaired cancer cells to DNA damaging agents and treatments. Also provided are methods for screening for compounds able to interact with, e.g., inhibit, enzymes involved in the G2 cell cycle arrest checkpoint, such as Chk1 and/or Chk2/Cds1 kinase.

WO 01/21771 A3

patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
14 February 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 00/01438

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N9/16 C07K14/16 A61K38/04 C12N15/11 C12N15/62
G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; SARKARIA, JANN N. ET AL: "Inhibition of ATM and ATR kinase activities by the radiosensitizing agent caffeine" retrieved from STN Database accession no. 131:283366 CA XP002181467 & CANCER RES. (1999), 59(17), 4375-4382 , abstract ---	1-86
A	WO 94 28914 A (MITOTIX) 22 December 1994 (1994-12-22) the whole document --- -/--	1-86

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 October 2001	08/11/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Masturzo, P

1

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 30680 A (PHARMACIA & UPJOHN) 16 July 1998 (1998-07-16) the whole document ---	1-86
P,X	M SUGANUMA ET AL.: "Sensitization of cancer cells to DNA damage-induced cell death by specific cell cycle G2 checkpoint abrogation" CANCER RESEARCH., vol. 59, 1 December 1999 (1999-12-01), pages 5887-5891, XP002181465 AMERICAN ASSOCIATION FOR CANCER RESEARCH, BALTIMORE, MD., US ISSN: 0008-5472 the whole document ---	1-86
A	X WANG ET AL.: "HIV-1 TAR RNA recognition by an unnatural biopolymer" JOURNAL OF THE AMERICAN CHEMICAL SOCIETY., vol. 119, no. 27, 1997, pages 6444-6445, XP002181466 AMERICAN CHEMICAL SOCIETY, WASHINGTON, DC., US ISSN: 0002-7863 the whole document -----	54

1

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-86

Present claims 1-86 relate to an extremely large number of possible compounds and methods based on them. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds and methods claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds prepared in the examples and methods based on them, and to the scope of claims 1-86 where the word "comprising" of claim 1 ff. is interpreted as "having".

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
Fu./IB 00/01438

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9428914	A	22-12-1994	US	5443962 A	22-08-1995
			AU	702174 B2	18-02-1999
			AU	7055194 A	03-01-1995
			EP	0708653 A1	01-05-1996
			JP	8510650 T	12-11-1996
			WO	9428914 A1	22-12-1994
			US	6251585 B1	26-06-2001
WO 9830680	A	16-07-1998	AU	6014998 A	03-08-1998
			WO	9830680 A2	16-07-1998